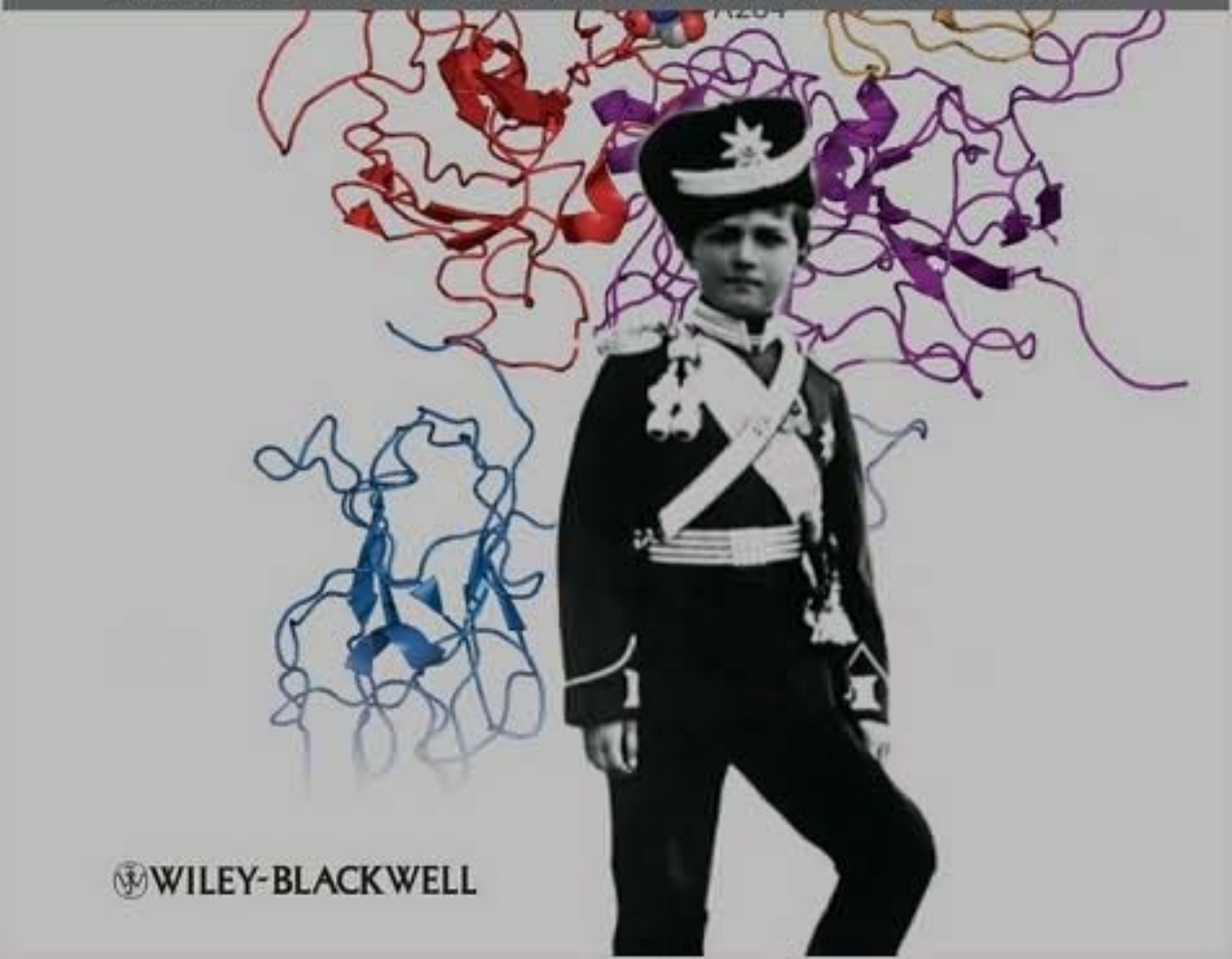
Textbook of Hemophilia

SECOND EDITION

EDITED BY CHRISTINE LEE, ERIK BERNTORP & KEITH HOOTS



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Second Edition

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Historical introduction

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It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of Light, it was the season of Darkness, it was the spring of hope, it was the winter of despair, we had everything before us, we had nothing before us, we were all going direct to Heaven, we were all going direct the other way.

Charles Dickens, A Tale of Two Cities *(1859)*

Early history

Hemophilia is probably one of the best examples in medicine where basic scientific discovery has been rapidly translated into clinical practice. Many patients with hemophilia have been enthusiastic to participate in trials of new treatments, and although such treatments have prolonged life, they have also been associated with devastating side-effects.

Many people have been aware of this rare sex-linked disorder because Queen Victoria, who reigned from 1837 to 1901, was a carrier [1] (Figure 1). She had two carrier daughters, Alice and Beatrice, and a son with hemophilia, Leopold [2]. Her daughter Alice was the grandmother of Alexis, the Tsarevich, whose repeated hemophilic bleedings resulted in his mother, Alexandra, coming under the influence of Rasputin. It has been suggested that hemophilia may have had a profound effect on Russian history [3]. Beatrice, born in 1856, was the last child of Victoria and Albert. Her daughter Ena became Queen of Spain and had two hemophilic sons, Alphonso and Gonzalo. Beatrice had three sons, two of whom, Leopold and Maurice, were affected with hemophilia. All three sons served during World War I, when Maurice was killed, but Leopold died in his late 20s following surgery [4].

Dr John Otto, a physician in the New York Hospital from 1796 to 1817, published the first medical description of hemophilia—this was a case of a woman carrier, and the sex-linked inheritance was noted as well as the occurrence of premature death [5].

Bulloch and Fildes' *Treasury of Human Inheritance* has been described "for students of haemophilia … at once [their] Shakespeare for its drama and human warmth and their bible for its towering authority" with 1000 references and case reports and 200 pedigrees of hemophilic families [1,6]. It includes a description of seven generations of the Appleton-Swain family, originating from a small town near Boston, USA, from the early part of the eighteenth century to the later years of the nineeteenth. This family was first described by Hay who noted, "None but males are bleeders … whose daughters only have sons thus disposed." The kindred was re-investigated by William Osler in 1885. Many of the hemophilic males died an early death from bleeding [6].

The natural history of hemophilia without treatment was vividly reported in a monograph published by Carroll Birch in 1937 from the USA. This was summarized by Biggs [7]. The cause of death in 113 patients was recorded—many died from very trivial injury; 82 died before 15 years of age and only eight survived beyond 40 years (Table 1).

Treatment

The first treatment for hemophilia was reported in 1840 in *The Lancet* [8]. George Firmin, an 11-year-old boy, bled after surgery for squint. Using the recently developed syringe by Dr. Blundell (Figure 2), blood from "a stout woman" was directly transfused and the child survived. The paper describes the inheritance of hemophilia in the family.

Fractionation of human plasma was developed in response to the challenges of World War II. The major components of plasma were separated by the control of their solubility in a multivariable system. The five variables were salt, protein, alcohol, pH, and temperature [9]. Cohn's fraction 1 was rich in factor VIII and fibrinogen.

McMillan pioneered the use of human factor VIII in the USA and in 1961 he published his experience [10]. Replacement therapy with Cohn's fraction 1 was used in 15 hemophilic patients presenting with a variety of hemorrhagic and surgical conditions. There was effective hemostasis in all patients. However, mild and transient hepatitis developed in one patient 35 days after infusion (this was possibly hepatitis C).

In 1954, in the UK, Macfarlane speculated that:
… maintenance therapy would be impracticable if only human AHG (FVIII) were available, since it would need a special panel of about 500,000 donors to treat the 500 hemophiliacs estimated to exist in the country (the UK) …

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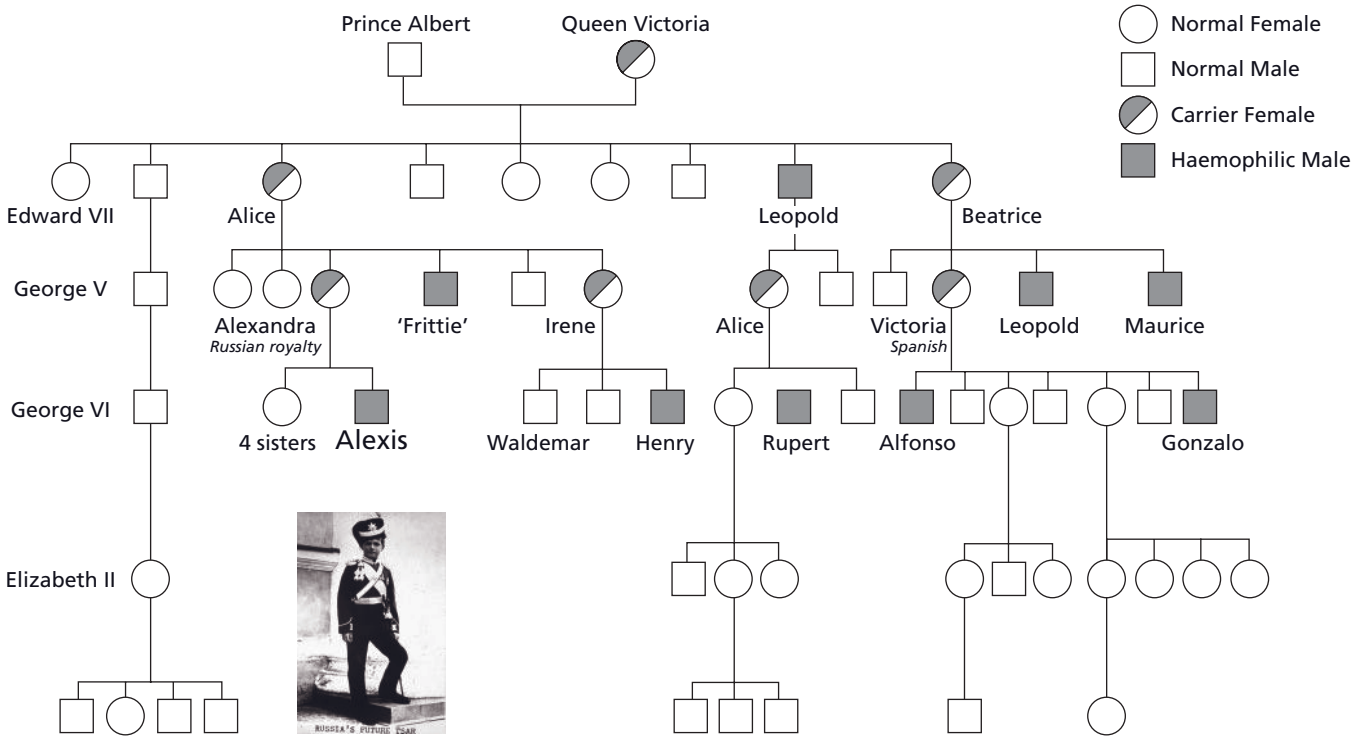

Figure 1 The family tree of Queen Victoria. Reproduced from [6] with permission.

Table 1 Cause of death for 113 cases of hemophilia by Carroll Birch.

Cause of death	No. of cases	Notes	
Operations	25	Circumcision	15
		Tooth extraction	6
		Vaccination	1
		Lanced hematomata	2
		Tonsillectomy	1
Trivial injuries	23	Cut lip, bitten tongue, injuries to forehead, finger, scalp, etc.	
Epistaxis	6	Four with serious injuries	
Hematuria	4		
Throat bleeding	3		
Cutting first tooth	1		
Fracture of leg	1		
Internal bleeding	21		
Central nervous bleeding	7		
Lung hemorrhage	5		
Intestinal bleeding	3		
Gastric bleeding	3		
Miscellaneous	4		
Birth trauma and umbilical bleeding	7		

Reproduced from [7] with permission.

Bovine blood has 16 times the anti haemophilic activity (FVIII) of human blood and enough would be available for the continuous treatment of the whole haemophilic population of this country [11].

Thus, bovine antihemophilic globulin (AHG, FVIII) was produced in Oxford, UK, and used to cover tooth extractions. The treatment was effective and the rise in FVIII was measured by the newly developed thromboplastin generation test [12]. However, the material showed some antigenic properties—an early recognition of inhibitor or antibody development. This led the Oxford group to develop an alternative animal source of FVIII—porcine FVIII [13].

The scientist Edith Bidwell led much of the early fractionation work at Oxford, and in 1961 the first patient to be treated with human FIX concentrate was reported [14]. A 4-year-old boy, with severe hemophilia B, had developed a large hematoma following a difficult venipuncture. The resulting hemorrhage had become infected resulting in osteomyelitis of the radius. A through-the-elbow amputation was performed in June 1960 under cover of FIX concentrate. The patient, aged 39 in 1995, qualified as an architectural technician, drove, and played golf [15].

The life of people with hemophilia was revolutionized by the development of cryoprecipitate. Judith Pool, in the USA, had discovered that if plasma was cooled to a very low temperature in the test tube a "cryoprecipitate" developed, which contained fibrinogen and FVIII. A method for making cryoprecipitate in a closed-bag system from a single blood dona-

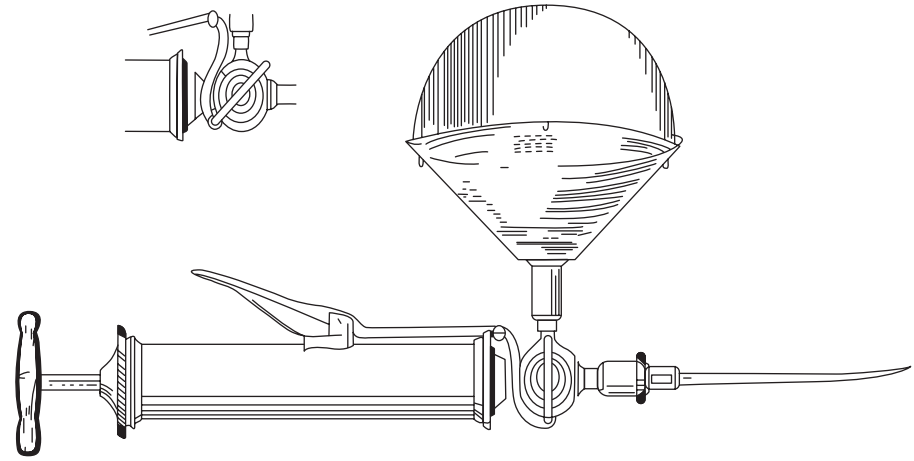

Figure 2 Blundell's syringe for the direct transfusion of blood. Reproduced from [8] with permission.

tion was described [16]. This meant that people with hemophilia could learn to treat themselves at home for the first time. Such treatment is still used in the developing world.

During the 1970s human freeze-dried (lyophilized) FVIII and FIX became available and patients were able to treat themselves more conveniently at home. The blood donors were British for the manufacture of NHS concentrates. Commercial products were manufactured from mostly American donor plasma. The donor pool size could be between 10 000 and 20 000 donations and the cryoprecipitate was produced from large-pool fresh-frozen plasma. The FVIII was extracted using ethanol and salt—Cohn's fractionation—and the final product was freeze dried or lyophilized. It was reconstituted by adding water and (self)-administered intravenously. Such products were not heated until 1985.

The availability of these products resulted in a dramatic increase in treatment. The lives of patients with hemophilia were improved because they could self-treat at home as soon as spontaneous bleeds occurred. However, they resulted in epidemics of human immunodeficiency virus (HIV) and hepatitis C virus (HCV).

Human immunodeficiency virus

The epidemic of HIV occurred during the years 1978–85 and was largely caused by USA-derived commercial concentrate. The first patient to seroconvert in the UK was treated in 1979 for abdominal bleeding and he developed non-A non-B hepatitis (HCV) followed by HIV [17]. When an HIV test became available in 1985 it was possible to retrospectively test stored samples from patients with hemophilia to establish the dates of seroconversion. In this way, a cohort of 111 patients with HIV with known dates of seroconversion was identified (Figure 3) [18]. The median age was 22 years (range 2–77) and the median date of infection was January 1983 (range December 1979 to July 1985). All these patients were coinfected with HCV either at or before the time of HIV infection.

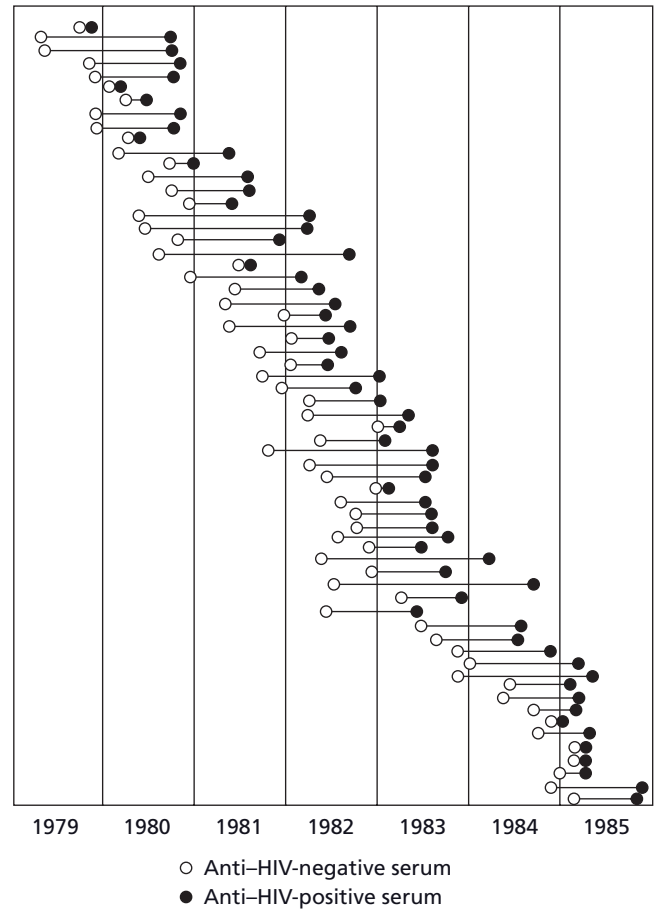

Figure 3 Patients with hemophilia and estimated dates of seroconversion. Reproduced from [18] with permission.

This cohort was closely monitored clinically, and serial CD4 counts were assessed regularly from December 1982. It was established that there was a linear decline of CD4 count from the normal of 800/μL and on average acquired immunodeficiency syndrome (AIDS) developed when the CD4 count

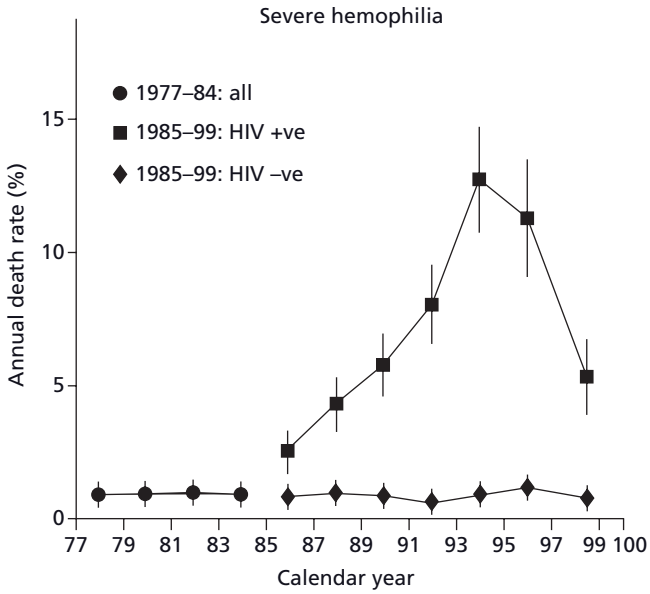

Figure 4 Impact of HIV on mortality rates in the UK hemophilia population. Reproduced from [25] with permission.

reached 50 [19]. It was thus possible to model for each patient in the cohort the time from seroconversion when he would develop AIDS [20]. Predictions from this study in 1994 suggested that one-fourth of people infected with HIV would remain free of AIDS (without treatment) for 20 years or more. At that time this aroused much publicity because previously it had been thought that HIV inevitably resulted in rapid death.

The epidemic of HIV in hemophilic patients in the USA showed an increase in deaths per million from 0.50 in the 1970s to 60 by 1990 [21]. In the UK, 1246 of 7250 patients with hemophilia were infected with HIV. Observations on this well-characterized cohort resulted in a series of publications charting the course of the epidemic [22–25]. Highly active antiretroviral therapy became available in the early 1990s and as a result the deaths from HIV were reduced. This was shown very clearly in the patients with hemophilia in the UK (Figure 4) [25].

Hepatitis C virus

The epidemic of HCV was a much longer one, from 1961 to 1985. The first patients became infected from the first large-pool plasma-derived FIX concentrates, used in 1961, and the epidemic ended with the dry heating of concentrates in 1985. Thus, all patients with HIV were coinfected with HCV either at the time of HIV infection or before. The natural history of HCV in a population of 310 patients whose date of infection was known showed that 25 years after infection with HCV 19% had progressed to death from liver disease and that HIV was a cofactor for progression [26].

However, the first recognition that hepatitis was a hazard of blood transfusion was a publication in 1943 [27], reporting seven cases of jaundice occurring 1–4 months after transfusion of whole blood or plasma, and a publication in 1946 [28], showing the increased risk of pooled plasma. Thus, it was not surprising that large-pool clotting factor concentrates should cause hepatitis; however, this was difficult to characterize in the absence of a test for HCV until 1991. There was also enthusiasm for the new concentrates among both patients and their treaters. In a historical interview Dr. Rosemary Biggs explained, "The next thing that started to crop up was that patients started to get jaundice, and we felt at that time that they were better alive and having jaundice than dead with haemophilia" [29]. In an anonymous leader it was recognized that "In some cases early death from liver disease may be the price paid by haemophiliacs for the improved quality of life afforded by the easy availability of clotting factor concentrates" [30].

The high risk, approaching 100%, of non-A non-B hepatitis following a first exposure to plasma-derived clotting factor concentrate (irrespective of whether the donors were of NHS or USA commercial origin) was demonstrated, although the hepatitis from commercial product was more severe, with a shorter incubation period [31]. Once testing had become available it was possible to characterize the HCV epidemic in hemophilic patients more clearly [26]. Approximately one-third of those infected with HCV were also infected with HIV. It was found that the relative hazard of death for those coinfected with HIV and HCV was 19.47 compared with those infected with HCV alone [26].

Many patients with hemophilia have been "cured" or "cleared" of HCV with interferon-based therapies, most recently with pegylated interferon and ribavirin. In an international multicenter cohort study, 147 patients maintained a sustained viral response up to 15 years after treatment whereas in 148 unsuccessfully treated patients the cumulative incidence of end-stage liver failure was 13% after 15 years [32].

The ultimate cure for end-stage liver failure is liver transplantation, and a small number of transplants have been performed in hemophilic patients. A report in 2002 described 11 hemophilic patients who were monoinfected with HCV and who had been successfully transplanted. Since the liver is the site of synthesis of clotting factors, on average, 36 hours post transplant the patients no longer needed treatment with clotting factor concentrate—liver transplantation is essentially "gene therapy" for hemophilia [33].

New products

The epidemics of HIV and HCV were the stimuli to achieve safe plasma-derived products using viral inactivation processes. These were effectively introduced in 1985 and no HIV or HCV transmissions following exposure to clotting factor concentrates have occurred since that time. The first-generation products were conventionally fractionated and heated in

lyophilized state ("dry heated"). These have now been withdrawn. Second-generation products involve dry superheating at 80°C for 72 h; solvent detergent; pasteurization; and heating in hot vapor. Third-generation products are prepared by monoclonal immunoadsorption directed to either FVIII or von Willebrand factor, the carrier protein for FVIII [34].

In 1984, a series of landmark papers were published in *Nature* describing the structure of FVIII and the cloning of the gene [35]. This enabled the manufacture of recombinant FVIII and the investigation of such products in worldwide trials. The results of a study in 107 patients, including pharmacokinetics, treatment for home therapy, surgery, and in previously untreated patients (PUPs), who were mostly children, demonstrated that it had biologic activity similar to plasma FVIII and was safe and efficacious in the treatment of hemophilia [36]. This meticulous study showed, for the first time, the natural history of the treatment in PUPs and the development of inhibitors (antibodies to FVIII)—6 of 21 children developed inhibitors. It soon emerged that the three recombinant products, two full-length FVIII, and one B-domain deleted, had similar inhibitor incidences of 25% [37,38]. Inhibitors have now emerged as the biggest challenge in the treatment of hemophilia.

Variant Creutzfeldt–Jakob disease

Even though plasma-derived concentrates are very safe with respect to HIV and hepatitis transmission, and also recombinant products are used predominantly in the developed world, there remains the possibility of variant Creutzfeldt–Jakob disease (vCJD), particularly in the UK.

The peak exposure of the UK population to vCJD through the food chain was in 1998 when nearly 400 000 cattle were infected with bovine spongiform encephalopathy (BSE). There has been almost no BSE since 2000 and the small epidemic of vCJD in humans has peaked with a total 166 cases (www.cjd.ed.ac.uk).

However, there have now been four cases of transmission by blood from donors incubating vCJD [39]. Thus, surveillance of the UK hemophilia population is ongoing because many were treated with plasma-derived concentrates manufactured from UK-derived plasma between 1980 (when the epidemic of BSE began) and 2001 (when concentrates derived from non-UK plasma were used exclusively) [40]. At the time of writing, abnormal prion protein has been demonstrated at post mortem in the splenic tissue of a patient with hemophilia who died from other causes [41].

The future

The outlook for people with hemophilia is now very good. In a study of 6018 people with hemophilia in the UK between 1977 and 1998, who were not infected with HIV, the median life expectancy was 63 years for those with severe hemophilia and 75 years for those with nonsevere hemophilia. This approaches that for the normal male population (Figure 5) [42].

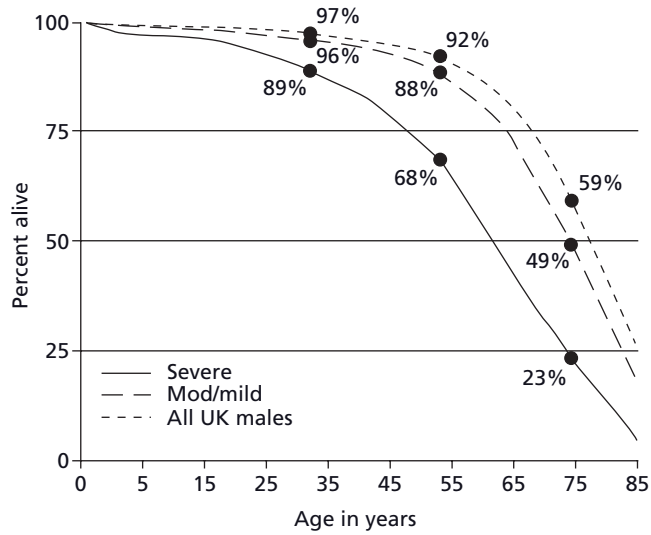

Figure 5 Survival in 6018 men with hemophilia not infected with HIV between 1977 and 1998 and in the general male UK population. Mod, moderate. Reproduced from [42] with permission.

This new edition of *The Textbook of Hemophilia* gives a perspective on the "state of the art" in 2010. There are still many challenges, but as the history of hemophilia shows, there is no doubt that future advances in basic scientific discovery will be rapidly translated into clinical practice.

References

1 Ingram GIC. The history of haemophilia. *J Clin Path* 1976; **29**: 469–79.
2 Massie RK. *Nicholas and Alexandra*. London: Gollanz, 1968.
3 Zeepvat C. *Prince Leopold: The untold story of Queen Victoria's Youngest Son*. Gloucester, UK: Sutton Publishing, 1998.
4 Dennison M. *The Last Princess*. London, UK: Weidenfeld and Nicolson, 2007.
5 Otto JC. *Review of American Publications in Medicine, Surgery and the Medical Repository*, **VI**(I): 1–4. New York: Auxillary Branches of Sciences, 1803.
6 Bulloch W, Fildes P. *Treasury of Human Inheritance*, Parts V and VI, Section XIVa, "Haemophilia". London: Dulan and Co., 1911.
7 Biggs R. Thirty years of haemophilia treatment in Oxford. *Br J Haematol* 1967; **13**: 452–63.
8 Lane S. Successful transfusion of blood. *Lancet* 1840; **i**:185–8.
9 Cohn EJ, Strong LE, Hughes WL, *et al*. Preparation and properties of serum and plasma proteins. *J Am Chem Soc* 1946; **68**: 459–75.
10 McMillan CW, Diamond LK, Surgenor DM. Treatment of classical haemophilia: The use of Fibrinogen Rich in FVIII for haemorrhage and surgery. *N Engl J Med* 1961; **265**: 224–30 and 277–83.

11 Macfarlane RG, Biggs R, Bidwell E. Bovine anti haemophilic globulin in the treatment of haemophilia. *Lancet* 1954; **i**: 1316–19.

12 Biggs R, Douglas AS. The thromboplastin generation test. *J Clin Path* 1953; **6**: 23.

13 Bidwell E. The purification of antihaemophilic globulin from animal plasma. *Br J Haematol* 1955; **1**: 35–45 and 386–9.

14 Biggs R, Bidwell E, Handley DA, *et al*. The preparation and assay of Christmas factor (factor IX) concentrate and its use in the treatment of two patients. *Br J Haematol* 1961; **7**: 349–64.

15 Rizza CR. Historical annotation: The first patient to receive factor IX concentrate in the UK: a recollection. *Haemophilia* 1995; **1**: 210–12.

16 Pool JGP, Shannon AE. Production of high-potency concentrates of antihaemophilic globulin in a closed-bag system. *N Engl J Med* 1965; **273**: 1443–7.

17 Lee CA, Webster A, Griffiths PD, Kernoff PBA. Symptomless HIV infection after more than ten years. *Lancet* 1990; **i**: 426.

18 Lee CA, Phillips A, Elford J, *et al*. The natural history of human immunodeficiency virus infection in a haemophilic cohort. *Br J Haematol* 1989; **73**: 228–34.

19 Phillips AN, Lee CA, Elford J, *et al*. Serial CD4 lymphocyte counts and the development of AIDS. *Lancet* 1991; **337**: 389–92.

20 Phillips AN, Sabin CA, Elford J, Bofill M, Janossy G, Lee CA. Use of CD4 lymphocyte count to predict long term survival free from AIDS after HIV infection. *BMJ* 1994; **309**: 309–13.

21 Chorba TL, Holman RC, Strine TW. Changes in longevity and causes of death among persons with haemophilia A. *Am J Haem* 1991; **37**: 243–6.

22 Darby SC, Rizza CR, Doll R, Spooner RJD, Stratton IM, Thakrar B. Incidence of AIDS and excess of mortality associated with HIV in haemophiliacs in the United Kingdom: report on behalf of the Directors of Haemophilia Centres in the UK. *BMJ* 1989; **298**: 1064–8.

23 Darby SC, Ewart W, Giangrande PLF, Dolin PJ, Spooner RJD, Rizza CR; on behalf of the UK Haemophilia Directors Organisation. Mortality before and after HIV infection in the complete UK population of haemophiliacs. *Nature* 1995; **377**: 79–82.

24 Darby SC, Ewart DW, Giangrande PLF, Spooner RJD, Rizza CR, for the UK Haemophilia Directors Organisation. Importance of age at infection with HIV-1 for survival and development of AIDS in the UK haemophilia population. *Lancet* 1996; **347**: 1573–80.

25 United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO). The impact of mortality rates in the complete UK haemophilia population. *AIDS* 2004; **18**: 525–33.

26 Yee TT, Griffioen A, Sabin CA, Dusheko G, Lee CA. The natural history of HCV in a cohort of haemophilic patients infected between 1961 and 1985. *Gut* 2000; **47**: 845–51.

27 Beeson PB. Jaundice occurring one to four months after transfusion of blood or plasma. *JAMA* 1943; **121**: 1332–4.

28 Spurling N, Shone J, Vaughan J. The incidence, incubation period and symptomatology of homologous serum jaundice. *BMJ* 1946; **2**: 409–11.

29 Historical Annotation. Witnessing medical history: an interview with Dr Rosemary Biggs. *Haemophilia* 1998; **4**: 769–77.

30 Leader. *Br Med J* 1981; **283**: 1.

31 Kernoff PBA, Lee CA, Karayiannis P, Thomas HC. High risk of non-A non-B hepatitis after a first exposure to volunteer or commercial clotting factor concentrates: effects of prophylactic immune serum globulin. *Br J Haematol* 1985; **60**: 469–79.

32 Posthouwer D, Yee TT, Makris M, *et al*. Antiviral therapy for chronic hepatitis C in patients with inherited bleeding disorders: an international, multicenter cohort study. *J Thromb Haemost* 2007; **5**: 1624–9.

33 Wilde J, Teixeira P, Bramhall SR, Gunson B, Multimer D, Mirza DF. Liver transplantation in haemophilia. *Br J Haematol* 2002; **117**: 952–6.

34 Lee CA. Coagulation factor replacement therapy. In: Hoffbrand AV, Brenner MK (eds) *Recent Advances in Haematology,* 6th edn. Edinburgh: Churchill Livingstone, 1992: 73–88.

35 Vehar GA, Key B, Eaton D, *et al*. Structure of human FVIII. *Nature* 1984; **312**: 337–42.

36 Scharz RS, Abildgaaard CF, Aledort LM, *et al*. Human recombinant DNA-derived antihaemophilic factor (factor VIII) in the treatment of haemophilia. *N Engl J Med* 1990; **323**: 1800–5.

37 Bray GL, Gompertz ED, Courter S, *et al*. A multicenter study of recombinant FVIII (Recombinant): safety, efficacy, and inhibitor risk in previously untreated patients with haemophilia A. *Blood* 1994; **83**: 2428–35.

38 Lusher JM, Lee CA, Kessler CM, Bedrosian CL, for the Refacto Phase 3 Study Group. The safety and efficacy of B-domain deleted recombinant factor VIII concentrate in patients with severe haemophilia A. *Haemophilia* 2003; **9**: 38–49.

39 Llewelyn CA, Hewitt PE, Knight RS, *et al*. Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. *Lancet* 2004; **363**: 417–21.

40 Hill FGH, Zaman A, Connor N, Millar C, Ironside J, Gill N. Extent of exposure to and risk stratification of UK patients treated with plasma concentrates with contribution from a donor who developed vCJD. Abstract OC-MO-034 XXII Congress ISTH. Boston, July 2009.

41 Ironside JW, Peden MW, Keeling DM, Millar CM, Hill FGH. Detection of PRPSC in the spleen of an adult haemophilic patient in the UK. Abstract OC-MO-032 XXII Congress ISTH. Boston, July 2009.

42 Darby SC, Kan SW, Spooner RJ, *et al*. Mortality rates, life expectancy, and causes of death in people with haemophilia A or B in the United Kingdom who were not infected with HIV. *Blood* 2007; **110**: 815–25.

The editors would like to express their gratitude to Professor Edward G.D. Tuddenham for permission to reproduce the cover image of factor IX and for the following legend. (CL, EB, KH)

The Tsarevitch Alexei Romanoff was a descendant of Queen Victoria who had severe haemophilia with bleeding from an early age. By the time of this photograph he already had a flexion deformity of his left hip and knee due to bleeding. As all descendants of Queen Victoria affected by her mutation had died before factor VIII and factor IX were differentiated, the type of haemophilia in the royal families of Europe was unknown until in 2009. Forensic DNA analysis of bones of the murdered Russian Royal family, recovered from graves near Yekaterinburg, prove that the royal disease was Haemophilia B, due to single base substitution creating a novel out of frame splice acceptor site at the 5′ end of intron 3. The molecule in the background is factor IX which Alexei lacked, causing the tragic bleeding that changed the course of history.

Reference: Genotype Analysis Identifies the Cause of the "Royal Disease" Evgeny I. Rogaev, Anastasia P. Grigorenko, Gulnaz Faskhutdinova, Ellen L. W. Kittler, Yuri K. Moliaka. *Science*: 326; **817** (2009).

1 Overview of hemostasis

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Introduction

The maintenance of blood fluidity and protection from blood leakage provide major biophysical challenges for the organism. Nature has evolved a highly complex, integrated, and dynamic system which balances the presentations of procoagulant, anticoagulant, and fibrinolytic systems. These systems function collectively to maintain blood within the vasculature in a fluid state while at the same time providing potent leak attenuating activity which can be elicited upon vascular perforation to provide the rapid assembly of a thrombus principally composed of platelets and fibrin to attenuate extravascular blood loss. The dynamic control of this system is such that the coagulation response is under the synergistic control of a variety of blood and vascular inhibitors, resulting in a process that is regionally restricted to the site of vascular damage and does not propagate throughout the vascular system. The rapid coagulation response is also tightly linked to the vascular repair process during which the thrombus is removed by the fibrinolytic system which also is activated regionally to provide clot removal coincident with vascular repair.

A list of important procoagulant, anticoagulant, and fibrinolytic proteins, inhibitors, and receptors can be seen in Table 1.1.

Importance of complex assembly to coagulation

Laboratory data combined with clinical pathology lead to the conclusion that the physiologically relevant hemostatic mechanism is primarily composed of three procoagulant vitamin K-dependent enzyme complexes (which utilize the proteases factor VIIa, factor IXa, and factor Xa) and one anticoagulant vitamin K-dependent complex (which utilizes the protease thrombin) [1,2] (Figure 1.1). These complexes—extrinsic factor Xase (tissue factor–factor VIIa complex), intrinsic factor Xase (factor VIIIa–factor IXa complex) [3], and the protein Case complex (thrombin–thrombomodulin) [4]—are each composed of a vitamin K-dependent serine protease, a cofactor protein and a phospholipid membrane; the latter provided by an activated or damaged cell. The membrane-binding properties of the vitamin K-dependent proteins are a consequence of the post-translational γ-carboxylation of these macromolecules [5]. The cofactor proteins are either membrane binding (factor Va, factor VIIIa), recruited from plasma, or intrinsic membrane proteins (tissue factor, thrombomodulin). Cofactor–protease assembly on membrane surfaces yields enhancements in the rates of substrate processing ranging from 10^5–10^9-fold relative to rates observed when the same reactions are limited to solution-phase biomolecular interactions between the individual proteases (factor VIIa, factor IXa, and factor Xa) and their corresponding substrates [6–8] (Figure 1.2a). Membrane binding, intrinsic to complex assembly, also localizes catalysis to the region of vascular damage. Thus, a system selective for regulated, efficient activity presentation provides for a regionally limited, vigorous arrest of hemorrhage.

Additional complexes associated with the "intrinsic" pathway are involved in the surface contact activation of blood [3]. However, the association of the contact-initiating proteins (factor XII, prekallikrein, high-molecular-weight kininogen) with hemorrhagic disease is uncertain [9].

Of equal importance to the procoagulant processes is regulation of anticoagulation by the stoichiometric and dynamic inhibitory systems. The effectiveness of inhibitory functions are far in excess of the potential procoagulant responses. These inhibitory processes provide activation thresholds, which require presentation of a limiting concentration of tissue factor prior to significant thrombin generation [10]. Antithrombin and tissue factor pathway inhibitor [11] are the primary stoichiometric inhibitors while the thrombin–thrombomodulin–protein C system (protein Case, Figure 1.1) is dynamic in its function.

Extrinsic pathway to blood coagulation

The initiating event in the generation of thrombin involves the binding of membrane-bound tissue factor with plasma factor VIIa [12]. The latter is present in blood at ~0.1 nM [~1–2% of the factor VII concentration (10 nM)] [13]. Plasma factor VIIa does not express proteolytic activity unless it is bound to tissue factor; thus factor VIIa at normal blood level has no significant activity toward either factor IX or factor X prior to its binding to tissue factor. The inefficient active site of

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Table 1.1 Procoagulant, anticoagulant, and fibrinolytic proteins, inhibitors, and receptors.

Protein	Molecular weight (kDa)	Plasma concentration		Plasma $t_{1/2}$ (days)	Clinical manifestation[a]		Functional classification
		nmol/L	μg/mL		H	T	
Procoagulant proteins and receptors							
Factor XII	80	500	40	2–3	–		Protease zymogen
HMW kininogen	120	670	80		–		Cofactor
LMW kininogen	66	1300	90				Cofactor
Prekallikrein	85/88	486	42				Protease zymogen
Factor XI	160	30	4.8	2.5–3.3	+/–		Protease zymogen
Tissue factor	44			N/A			Cell-associated cofactor
Factor VII	50	10	0.5	0.25	+	+/–	VKD protease zymogen
Factor X	59	170	10	1.5	+		VKD protease zymogen
Factor IX	55	90	5	1	+		VKD protease zymogen
Factor V	330	20	6.6	0.5	+	+	Soluble pro-cofactor
Factor VIII	285	0.7	0.2	0.3–0.5	+	–	Soluble pro-cofactor
VWF	255 (monomer)	Varies	10		+		Platelet adhesion carrier for FVIII
Factor II	72	1400	100	2.5	+	–	VKD protease zymogen
Fibrinogen	340	7400	2500	3–5	+	+/–	Structural protein cell adhesion
Factor XIII	320	94	30	9–10	+	+/–	Transglutaminase zymogen
Anticoagulant proteins, inhibitors, and receptors							
Protein C	62	65	4	0.33		+	Proteinase zymogen
Protein S	69	300	20	1.75		+	Inhibitor/cofactor
Protein Z	62	47	2.9	2.5	+/–		Cofactor
Thrombomodulin	100	N/A	N/A	N/A			Cofactor/modulator
Tissue factor pathway inhibitor	40	1–4	0.1	6.4×10^{-4} to1.4×10^{-3}			Proteinase inhibitor
Antithrombin	58	2400	140	2.5–3			Proteinase inhibitor
Heparin cofactor II	66	500–1400	33–90	2.5	+	+/–	Proteinase inhibitor
Fibrinolytic proteins, inhibitors, and receptors							
Plasminogen	88	2000	200	2.2			Proteinase zymogen
t-PA	70	0.07	0.005	0.00167			Proteinase zymogen
u-PA	54	0.04	0.002	0.00347			Proteinase zymogen
TAFI	58	75	4.5	0.00694		+	Carboxypeptidase
PAI-1	52	0.2	0.01	<0.00694			Proteinase inhibitor
PAI-2	47/60	<0.070	<0.005	–			Proteinase inhibitor
α-Antiplasmin	70	500	70	2.6	+		Proteinase inhibitor
u-PAR	55						Cell membrane receptor

+, presence of phenotype; –, absence of phenotype; ±, some individuals present with the phenotype and others do not; H, hemorrhagic disease/hemophilia; T, thrombotic disease/thrombophilia; VKD; vitamin K-dependent proteins.

[a]Clinical phenotype; the expression of either hemorrhagic or thrombotic phenotype in deficient individuals.

factor VIIa permits its escape from inhibition by the antithrombin present in blood. Vascular damage [14] or cytokine-related presentation of the active tissue factor triggers the process by interaction with activated factor VIIa, which increases the k_{cat} of the enzyme and increases the rate of factor X activation by four orders of magnitude [15]. This increase is the result of the improvement in catalytic efficiency and the membrane binding of factor IX and factor X.

The tissue factor–factor VIIa complex (extrinsic factor Xase) (Figure 1.2) catalyzes the activation of both factor IX and factor X, the latter being the more efficient substrate [16]. Thus, the initial product formed is factor Xa. Feedback cleavage of factor IX by membrane-bound factor Xa enhances the rate of generation of factor IXa in a cooperative process with the tissue factor–factor VII complex [17].

The initially formed, membrane-bound factor Xa activates small amounts of prothrombin to thrombin [18]. This initial prothrombin activation provides the thrombin essential to the acceleration of the hemostatic process by serving as the activator for platelets [19], factor V [20], and factor VIII [21]

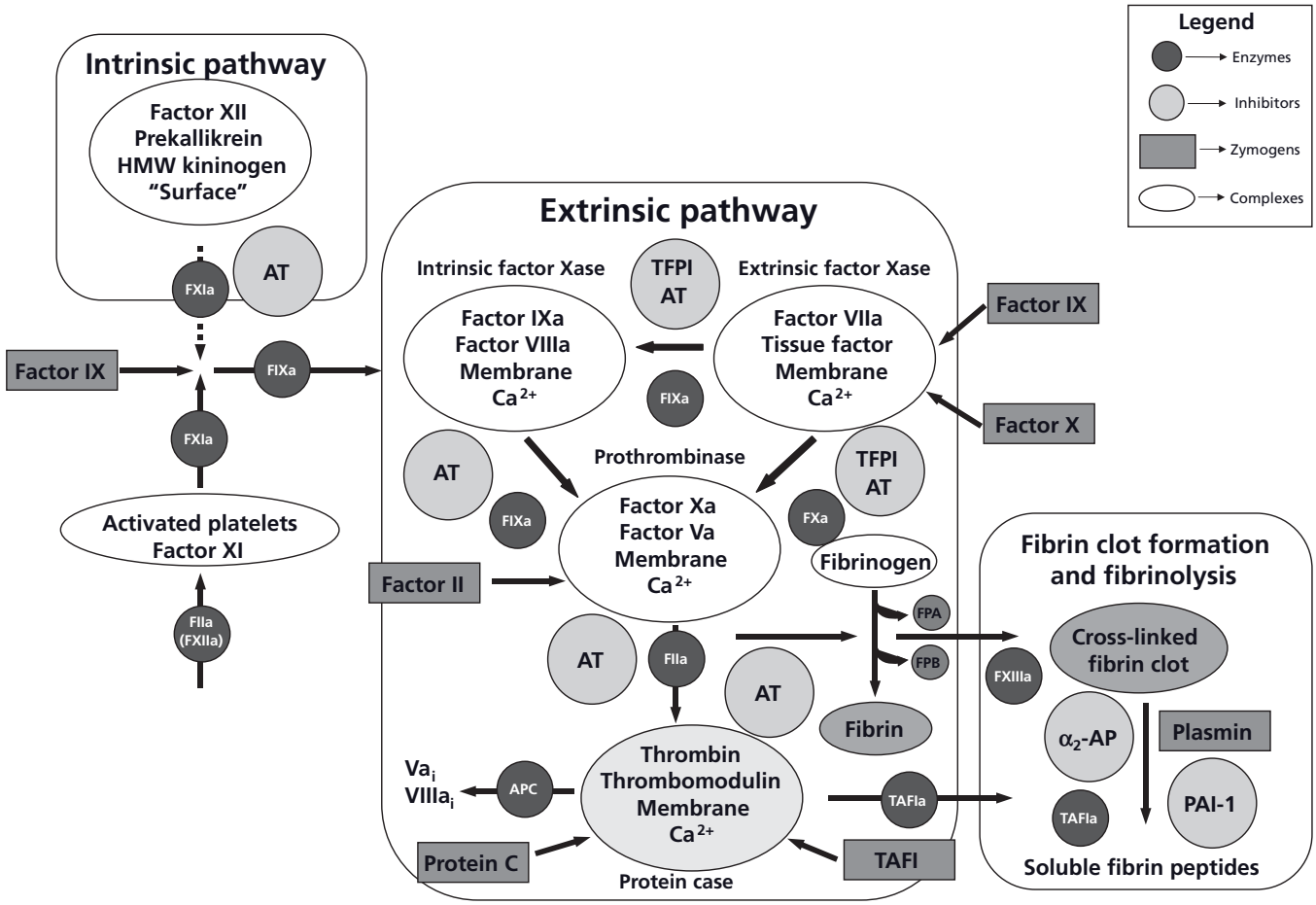
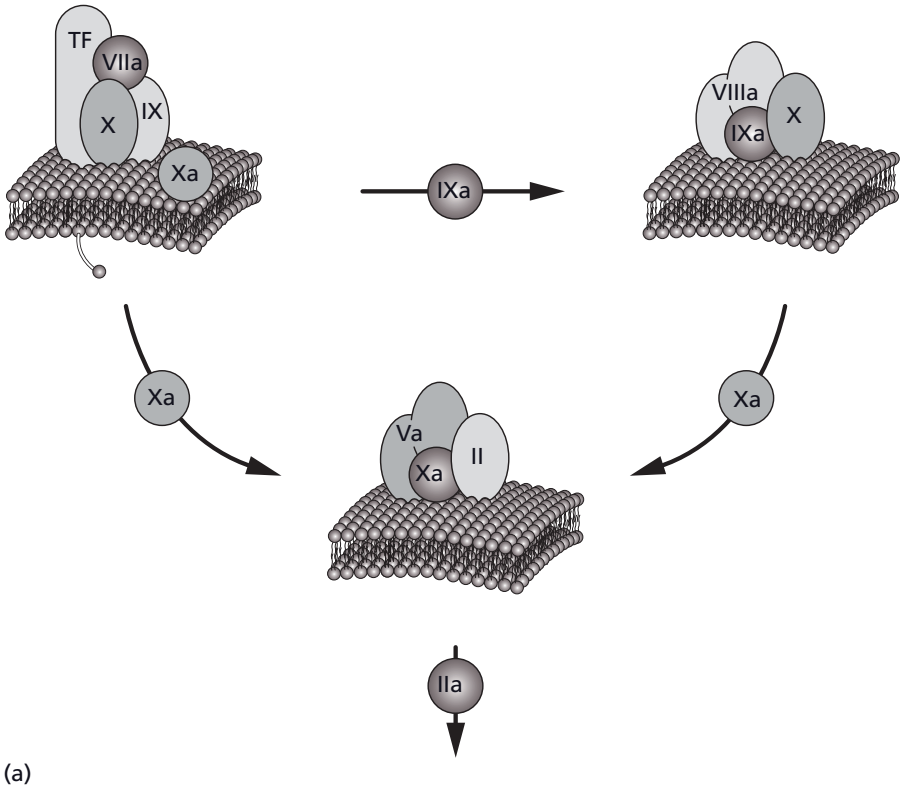


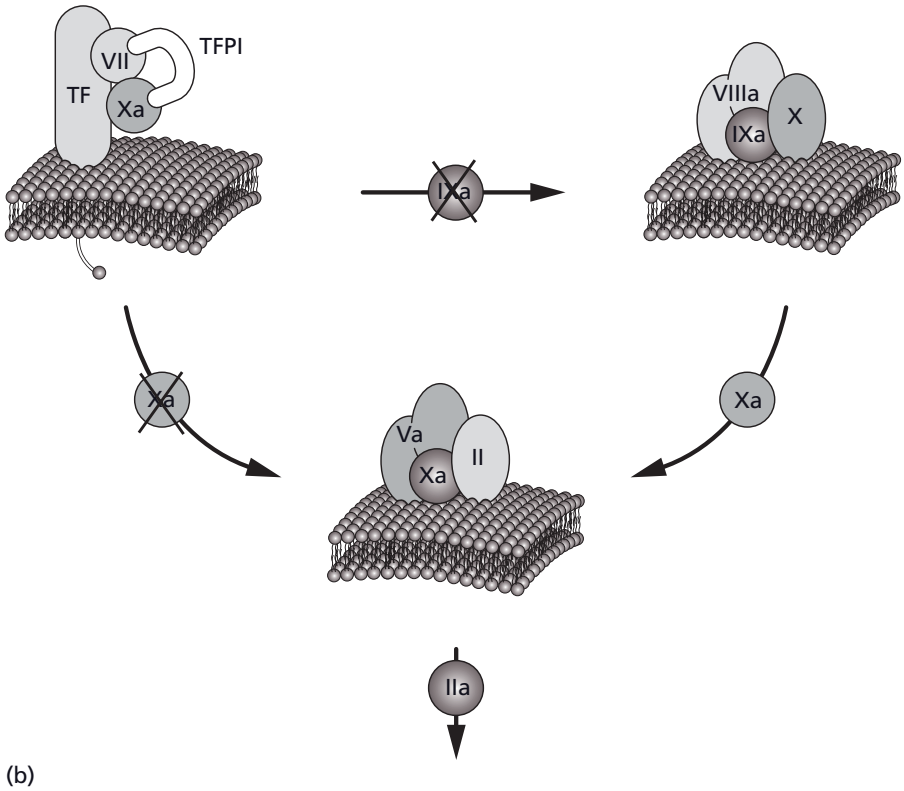
Figure 1.1 Overview of hemostasis. Coagulation is initiated through two pathways: the primary extrinsic pathway (shown on the right) and the intrinsic pathway (historically called the *contact* or *accessory pathway*, shown on the left). The components of these multistep processes are illustrated as follows: enzymes (*open circle*), inhibitors (*hatched circles*), zymogens (*open boxes*), or complexes (*open ovals*). Fibrin formation is also shown as an oval. The intrinsic pathway has no known bleeding etiology associated with it, thus this path is considered accessory to hemostasis. Upon injury to the vessel wall, tissue factor, the cofactor for the extrinsic factor Xase complex, is exposed to circulating factor VIIa and forms the vitamin K-dependent complex, the extrinsic factor Xase. Factor IX and factor X are converted to their serine proteases factor IXa (FIXa) and factor Xa (FXa), which then form the intrinsic factor Xase and the prothrombinase complexes, respectively. The combined actions of the intrinsic and extrinsic factor Xase and the prothrombinase complexes lead to an explosive burst of the enzyme thrombin (IIa). In addition to its multiple procoagulant roles, thrombin also acts in an anticoagulant capacity when combined with the cofactor thrombomodulin in the protein Case complex. The product of the protein Case reaction, activated protein C (aPC), inactivates the cofactors factors Va and VIIIa. The cleaved species, factors Va_i and $VIIIa_i$, no longer support the respective procoagulant activities of the prothrombinase and intrinsic Xase complexes. Once thrombin is generated through procoagulant mechanisms, thrombin cleaves fibrinogen, releasing fibrinopeptide A and B (FPA and FPB) and activate factor XIII to form a cross-linked fibrin clot. Thrombin–thrombomodulin also activates thrombin activatable fibrinolysis inhibitor (TAFIa) that slows down fibrin degradation by plasmin. The procoagulant response is downregulated by the stoichiometric inhibitors tissue factor pathway inhibitor (TFPI) and antithrombin (AT). TFPI serves to attenuate the activity of the extrinsic factor Xase trigger of coagulation. Antithrombin directly inhibits thrombin, factor IXa and factor Xa. The intrinsic pathway provides an alternative route for the generation of factor IXa. Thrombin has also been shown to activate factor XI. The fibrin clot is eventually degraded by plasmin-yielding soluble fibrin peptides. Modified from [32].

(Figure 1.1). Once factor VIIIa is formed, the factor IXa generated by tissue factor–factor VIIa combines with factor VIIIa on the activated platelet membrane to form the "intrinsic factor Xase" (Figure 1.2a), which becomes the major activator of factor X. The factor VIIIa–factor IXa complex is 10^9-fold more active as a factor X activator and 50 times more efficient than tissue factor–factor VIIa in catalyzing factor X activation [22,23]; thus, the bulk of factor Xa is ultimately produced by the factor VIIIa–factor IXa complex (Figure 1.2).

As the reaction progresses, factor Xa generation by the more active "intrinsic factor Xase" complex exceeds that of the "extrinsic factor Xase" complex [24]. In addition, the "extrinsic factor Xase" complex is subject to inhibition by tissue factor pathway inhibitor (Figure 1.2b) [25]. As a



(a)



(b)

Figure 1.2 Vitamin K-dependent complex assembly. (a) The factor Xa generated by the tissue factor–factor VIIa complex activates a small amount of thrombin which activates factor V and factor VIII leading to the presentation of the intrinsic factor Xase (factor VIIIa–factor IXa) and prothrombinase (factor Va–factor Xa) complexes. At this point in the reaction factor IXa generation is cooperatively catalyzed by membrane-bound factor Xa and by the tissue factor–factor VIIa complex. The thick arrow representing factor Xa generation by the intrinsic factor Xase illustrates the more efficient factor Xa generation by this catalyst. (b) The tissue factor pathway inhibitor (TFPI) interacts with the tissue factor–factor VIIa–factor Xa product complex to block the tissue factor-initiated activation of both factors IX and factor X. Inhibition of the extrinsic factor Xase complex results in the factor VIIIa–factor IXa complex (intrinsic factor Xase), becoming the only viable catalyst for factor X activation. Used with permission from the "Dynamics of Hemostasis" Haematologic Technologies, K.G. Mann, 2002.

consequence, most (>90%) of factor Xa is ultimately produced by the factor VIIIa–factor IXa complex in the tissue factor-initiated hemostatic processes. In hemophilia A and hemophilia B the "intrinsic factor Xase" complex cannot be assembled, and amplification of factor Xa generation does not occur [26]. Factor Xa combines with factor Va on the activated platelet membrane receptors and this factor Va–factor Xa "prothrombinase" catalyst (Figure 1.2a) converts prothrombin to thrombin. Prothrombinase is 300 000-fold more active than factor Xa alone in catalyzing prothrombin activation [6].

Attenuation of the procoagulant response

The coagulation system is tightly regulated by the inhibition systems. The tissue factor concentration threshold for reaction initiation is steep and the ultimate amount of thrombin produced is largely regulated by the concentrations of plasma procoagulants and the stoichiometric inhibitors and the constituents of the dynamic inhibition processes [24]. Tissue factor pathway inhibitor blocks the tissue factor–factor VIIa–factor Xa product complex, thus effectively neutralizing the "extrinsic factor Xase" complex (Figure 1.2b) [27]. However, tissue factor pathway inhibitor is present at low abundance (~2.5 nM) in blood and can only delay the hemostatic reaction [28]. Antithrombin, normally present in plasma at twice the concentration (3.2 μM) of any potential coagulation enzyme, neutralizes all the procoagulant serine proteases primarily in the uncomplexed state [11].

The dynamic protein C system is activated by thrombin binding to constitutive vascular thrombomodulin (Protein Case). This complex activates protein C to its activated species activated protein C (Figure 1.1) [4]. Activated protein C competes in binding with factor Xa and factor IXa and cleaves factor Va and factor VIIIa, eliminating their respective complexes [20]. The protein C system, tissue factor pathway inhibitor, and activated protein C cooperate to produce steep tissue factor concentration thresholds, acting like a digital "switch," allowing or blocking thrombin formation [10].

In humans, the zymogen factor XI which is present in plasma and platelets has been variably associated with hemorrhagic pathology [29]. Factor XI is a substrate for thrombin and has been invoked in a "revised pathway of coagulation" contributing to factor IX activation (Figure 1.1) [30]. The importance of the thrombin activation of factor XI is evident only at low tissue factor concentrations [26].

Factor XII, prekallekrein, and high-molecular-weight kininogen (Figure 1.1) do not appear to be fundamental to the process of hemostasis [31]. The contribution of these contact pathway elements to thrombosis remains an open question and further experimentation is required to resolve this issue [31].

Conclusion

Advances in genetics, protein chemistry, bioinformatics, physical biochemistry, and cell biology provide an array of information with respect to normal and pathologic processes leading to hemorrhagic or thrombotic disease. The challenge for the 21st century will be to merge mechanism-based, quantitative data with epidemiologic studies and subjective clinical experience associated with the tendency to bleed or thrombose and with the therapeutic management of individuals with thrombotic or hemorrhagic disease. *In vitro* data and clinical experience with individuals with thrombotic and hemorrhagic disease will ultimately provide algorithms which can combine the art of clinical management with the quantitative science available to define the phenotype *vis á vis* the outcome of a challenge or the efficacy of an intervention [28–34].

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References

1 Mann KG, Nesheim ME, Church WR, Haley P, Krishnaswamy S. Surface-dependent reactions of the vitamin K-dependent enzyme complexes. *Blood* 1990; **76**: 1–16.
2 Brummel-Ziedins K, Orfeo T, Jenny NS, Everse SJ, Mann KG. Blood coagulation and fibrinolysis. In: Greer JP, Foerster J, Lukens J, Rodgers GM, Paraskevas F, Glader B (eds.) *Wintrobe's Clinical Hematology*. Philadelphia: Lippincott Williams & Wilkins, 2003: 677–774.
3 Davie EW, Ratnoff OD. Waterfall sequence for intrinsic blood clotting. *Science* 1964; **145**: 1310–12.
4 Esmon CT. The protein C pathway. *Chest* 2003; **124**: 26S–32S.
5 Stenflo J. Contributions of Gla and EGF-like domains to the function of vitamin K-dependent coagulation factors. *Crit Rev Eukaryot Gene Expr* 1999; **9**: 59–88.
6 Nesheim ME, Taswell JB, Mann KG. The contribution of bovine Factor V and Factor Va to the activity of prothrombinase. *J Biol Chem* 1979; **254**: 10952–62.
7 van Dieijen G, Tans G, Rosing J, Hemker HC. The role of phospholipid and factor VIIIa in the activation of bovine factor X. *J Biol Chem* 1981; **256**: 3433–42.
8 Komiyama Y, Pedersen AH, Kisiel W. Proteolytic activation of human factors IX and X by recombinant human factor VIIa: effects of calcium, phospholipids, and tissue factor. *Biochemistry* 1990; **29**: 9418–25.
9 Davie EW. A brief historical review of the waterfall/cascade of blood coagulation. *J Biol Chem* 2003; **278**: 50819–32.
10 van't Veer C, Golden NJ, Kalafatis M, Mann KG. Inhibitory mechanism of the protein C pathway on tissue factor-induced thrombin generation. Synergistic effect in combination with tissue factor pathway inhibitor. *J Biol Chem* 1997; **272**: 7983–94.
11 Olson ST, Bjork I, Shore JD. Kinetic characterization of heparin-catalyzed and uncatalyzed inhibition of blood coagulation proteinases by antithrombin. *Methods Enzymol* 1993; **222**: 525–59.

12 Nemerson Y. Tissue factor and hemostasis. *Blood* 1988; **71**: 1–8.
13 Morrissey JH, Macik BG, Neuenschwander PF, Comp PC. Quantitation of activated factor VII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activation. *Blood* 1993; **81**: 734–44.
14 Osterud B. The role of platelets in decrypting monocyte tissue factor. *Semin Hematol* 2001; **38**: 2–5.
15 Bom VJ, Bertina RM. The contributions of Ca^{2+}, phospholipids and tissue-factor apoprotein to the activation of human blood-coagulation factor X by activated factor VII. *Biochem J* 1990; **265**: 327–36.
16 Osterud B, Rapaport SI. Activation of factor IX by the reaction product of tissue factor and factor VII: additional pathway for initiating blood coagulation. *Proc Natl Acad Sci USA* 1977; **74**: 5260–64.
17 Lawson JH, Mann KG. Cooperative activation of human factor IX by the human extrinsic pathway of blood coagulation. *J Biol Chem* 1991; **266**: 11317–27.
18 Butenas S, DiLorenzo ME, Mann KG. Ultrasensitive fluorogenic substrates for serine proteases. *Thromb Haemost* 1997; **78**: 1193–201.
19 Brass LF. Thrombin and platelet activation. *Chest* 2003; **124**: 18S–25S.
20 Mann KG, Kalafatis M. Factor V: a combination of Dr Jekyll and Mr Hyde. *Blood* 2003; **101**:20–30.
21 Fay PJ. Subunit structure of thrombin-activated human factor VIIIa. *Biochim Biophys Acta* 1988; **952**: 181–90.
22 Mann KG, Krishnaswamy S, Lawson JH. Surface-dependent hemostasis. *Semin Hematol* 1992; **29**: 213–26.
23 Ahmad SS, Rawala-Sheikh R, Walsh PN. Components and assembly of the factor X activating complex. *Semin Thromb Hemost* 1992; **18**: 311–23.
24 Hockin MF, Jones KC, Everse SJ, Mann KG. A model for the stoichiometric regulation of blood coagulation. *J Biol Chem* 2002; **277**: 18322–33.
25 Girard TJ, Warren LA, Novotny WF, Likert KM, Brown SG, Miletich JP, Broze GJ, Jr. Functional significance of the Kunitz-type inhibitory domains of lipoprotein-associated coagulation inhibitor. *Nature* 1989; **338**: 518–20.
26 Cawthern KM, van't Veer C, Lock JB, DiLorenzo ME, Branda RF, Mann KG. Blood coagulation in hemophilia A and hemophilia C. *Blood* 1998; **91**: 4581–92.
27 Baugh RJ, Broze GJ, Jr, Krishnaswamy S. Regulation of extrinsic pathway factor Xa formation by tissue factor pathway inhibitor. *J Biol Chem* 1998; **273**: 4378–4386.
28 Novotny WF, Brown SG, Miletich JP, Rader DJ, Broze GJ, Jr. Plasma antigen levels of the lipoprotein-associated coagulation inhibitor in patient samples. *Blood* 1991; **78**: 387–93.
29 Seligsohn U. Factor XI deficiency. *Thromb Haemost* 1993; **70**: 68–71.
30 Gailani D, Broze GJ, Jr. Factor XI activation in a revised model of blood coagulation. *Science* 1991; **253**: 909–12.
31 Colman RW. Contact activation pathway: Inflammatory, fibrinolytic, anticoagulant, antiadhesive and antiangiogenic activities. In: Colman RW, Hirsh J, Marder VJ, Clowes AW, George JN (eds.) *Hemostasis and Thrombosis: Basic Principles & Clinical Practice*. Philadelphia: Lippincott Williams & Wilkins, 2001: 103–121.
32 Undas A, Brummel-Ziedins KE, Mann KG. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. *Blood* 2007; **109**: 2285–92.
33 Brummel-Zeidins K, Vossen CY, Rosendaal FR, Umezaki K, Mann KG. The plasma hemostatic proteome: thrombin generation in healthy individuals. *J Thromb Haemost* 2005; **3**: 1472–81.
34 Mann GK, Brummel-Ziedins K, Undas A, Butenas S. Does the genotype predict the phenotype? Evaluations of the hemostatic proteome. *J Thromb Haemost* 2004; **2**: 1727–34.

2 Cellular processing of factor VIII and factor IX

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Factor VIII

Factor VIII and hemophilia A

Blood coagulation is a finely controlled process which enables plasma and cellular blood components to perform their functions in a fluid phase. However, upon damage to the lining of a blood vessel an insoluble clot must be formed at the site of injury to minimize loss of blood components. This process is initiated by activation of platelets and the formation of a primary platelet plug followed by the coordinated and highly regulated formation of a stable fibrin mesh. Maintenance of hemostasis relies on the regulated interaction of the vitamin K-dependent proteases, protease cofactors, membrane surfaces and receptors, calcium ions, and protease inhibitors. This process is characterized by the rapid and sequential activation of three separate vitamin K-dependent serine proteases—factors VII, factor IX, and factor X—and their cofactor complexes—tissue factor, factor VIII, and factor V—that make up the intrinsic, extrinsic, and common coagulation pathways, respectively. These pathways act to rapidly and efficiently cleave the vitamin K-dependent zymogen prothrombin to its active serine protease form, thrombin, at the site of injury, which leads to cleavage of soluble fibrinogen to insoluble fibrin and clot formation. Factor VIII acts as an essential cofactor for factor IX in the intrinsic coagulation cascade, amplifying factor IX activity by several orders of magnitude. The physiologic significance of these pathways is evident from genetic deficiencies that result in bleeding disorders. In the absence of factor VIII clot formation is impaired leading to prolonged bleeding. Mutations in *F8*, the gene coding for coagulation FVIII leading to deficiency of factor VIII or impaired factor VIII function, result in the clinical disease hemophilia A. Hemophilia A has been recognized for over 2000 years as an X-linked bleeding disorder characterized by spontaneous bleeding into joints and muscles and severe bleeding from traumas. Treatment of hemophilia A has steadily improved since the discovery in the nineteenth century that whole-blood transfusion improved coagulation in patients with hemophilia. In the 1980s the gene for factor VIII was cloned and this discovery led quickly to production of recombinant factor VIII in mammalian cells for replacement therapy in patients. All the proteins involved in the coagulation cascade require post-translational modifications for appropriate secretion, plasma half-life, and function-recombinant DNA technology has provided the ability to produce safe and efficacious preparations of factor VIII replacement therapy. Gene therapy approaches for hemophilia are rapidly approaching, and need to consider, the requirement for proper post-translational modification in protein secretion and function.

Domain structure of factor VIII

Factor VIII and factor V are homologous glycoproteins that serve as cofactors for proteolytic activation of factor X and prothrombin, respectively. These cofactors act to increase the V_{max} of substrate activation by four orders of magnitude. They have a conserved domain organization of A1–A2–B–A3–C1–C2 (Figure 2.1) [1]. The A domains of factors V and VIII are homologous to the A domains of the plasma copper-binding protein ceruloplasmin. Copper has been detected in factor VIII and its presence is associated with functional factor VIII activity [2]. One mole of reduced Cu(I) was detected in recombinant factor VIII and likely resides within a type 1 copper ion-binding site within the A1 domain [3]. The C domains are homologous to phospholipid-binding proteins such as milk-fat globule protein, suggesting a role in phospholipid interaction. While the amino acid sequences in the A and C domains are 40% identical between factors V and VIII, there is only limited homology between the B domains. However, the B domains of both proteins have conserved the addition of a large number of asparagine-linked oligosaccharides as well as a large number of serine/threonine-linked oligosaccharides, suggesting a role of the carbohydrate in cofactor function.

Recently, the crystal structure of a B-domain-less factor VIII was solved revealing a triangular heterotrimer composed of the three A domains with the A1 domain interacting with the C2 domain and the A3 domain interacting with the C2 domain [4,5]. This crystal structure and biochemical studies have yielded an *in silico* model of the activated factor VIII–activated factor IX complex with factor IXa wrapping across the side of factor VIIIa and forming an extended area of interaction

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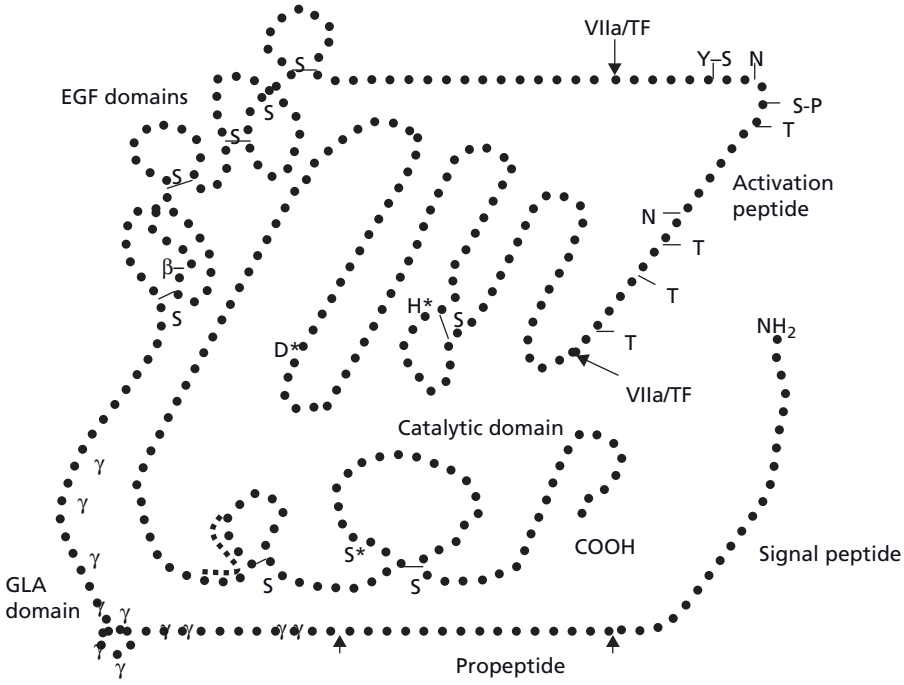

Figure 2.1 Domain structure and processing of factor VIII. The structural domains of factor VIII are depicted: A1 domain (1–336), A2 domain (372–740), B domain (740–1648), A3 domain (1690–2020) and the C domains (2021–2332). Above, the pairing of disulfide bonds is shown. Below are represented the potential N-linked glycosylation sites (vertical bars up). Three regions (stippled areas) rich in acidic amino acid residues and lying between domains A1 and A2, A2 and B, B and A3 contain sites of tyrosine sulfation (s). Intracellularly, factor VIII is cleaved within the B domain after Arg1313 and Arg1648 to generate an approximately 200-kDa peptide and the 80-kDa light chain. The two cleavages required for thrombin activation are indicated (**). The sites for aPC cleavage and inactivation are also shown (*).

including large portions of both the heavy and light chains of factor VIII [4]. Interestingly, these factor VIII structures contain two Cu^{2+} ions and one or two Ca^{2+} ions and three asparagine-linked carbohydrate moieties which are essential to the structure [4,5].

Factor VIII contains a 19-amino-acid signal peptide that is removed upon translocation into the endoplasmic reticulum (ER). Factor V is secreted from hepatocytes as a single-chain polypeptide of 330 kDa. Factor VIII is processed within the secretory pathway in the cell to yield a heterodimer primarily composed of a heavy chain extending up to 200 kDa (primarily two species from residues 1 to 1313 or 1648, where residue 1 is the amino-terminal amino acid after signal peptide cleavage) in a metal ion-dependent association with an 80-kDa light chain (residues 1649 to 2332) (Figure 2.2). This association is stabilized by noncovalent interactions between the amino-terminal and carboxy-terminal ends of the factor VIII light chain with the amino-terminus of mature von Willebrand factor (VWF). VWF interaction stabilizes factor VIII upon secretion from the cell, inhibits factor VIII binding to phospholipids, and increases the half-life of factor VIII circulating in plasma [6,7]. The ratio of VWF to factor VIII is maintained at 50:1, where an increase or decrease in the plasma VWF level results in a corresponding change in the level of factor VIII.

Factor V and factor VIII circulate in plasma as inactive precursors that are activated through limited proteolysis by either thrombin or activated factor X (Xa). Thrombin activation of factor VIII results in cleavage initially after Arg740 and subsequently after Arg residues 372 and 1689 [8]. Cleavage at both Arg372 and Arg1689 is required for activation of factor VIII procoagulant activity. The cleavage at Arg1689 releases activated factor VIII from VWF, thereby relieving the inhibitory activity of VWF on factor VIII, permitting the activated form of factor VIII to interact with negatively charged phospholipids. Thrombin-activated factor VIII consists of a heterotrimer of a 50-kDa A1-domain-derived polypeptide, a 43-kDa A2-domain-derived polypeptide, and a 73-kDa derived light-chain fragment [9,10]. Upon thrombin activation, the B domains of both factors V and VIII are released. The amino-terminal sides of the thrombin cleavage sites within factors V and VIII are rich in acidic amino acids and contain the post-translationally modified amino acid, tyrosine sulfate.

Disulfide bond formation

Factor VIII and factor V also have a conserved disulfide bonding pattern in which two disulfide bonds occur within the A1 and A2 domains, whereas only the small disulfide loop is present in their A3 domains. In addition, each C domain in factor V and VIII contains one disulfide bond [11]. There are a number of nondisulfide-bonded cysteine residues within factor VIII; one cysteine residue is not oxidized in each A domain and there are four cysteine residues within the B domain that are also likely not oxidized. Disulfide bond formation occurs in the oxidizing environment of the ER and it is possible that protein chaperones, such as protein disulfide isomerase, are important to ensure proper disulfide bond formation and exchange occurs prior to exit from the ER. Factor VIII contains a total of eight disulfide bonds, seven of which, interestingly, are found in factor V [12]. Replacement of cysteine residues with glycine in any of the seven conserved

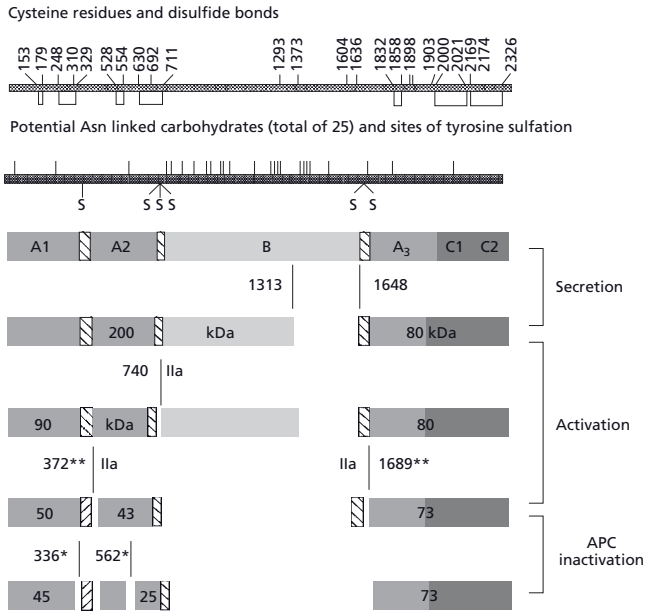

Figure 2.2 Synthesis, processing, and secretion of factor VIII in mammalian cells. The factor VIII primary translation product is translocated into the lumen of the endoplasmic reticulum (ER), where N-linked glycosylation occurs. A fraction of factor VIII binds tightly to the protein chaperone BiP and requires adenosine triphosphate (ATP) hydrolysis for release [43]. A portion of factor VIII is retrotranslocated into the cytoplasm and is degraded by the cytosolic 26S proteasome. Another fraction of the molecules interact with the lectins calnexin/calreticulin and then with the protein chaperone complex LMAN1–MCFD2 for transit to the Golgi apparatus. In the Golgi apparatus additional processing occurs that includes complex modification of carbohydrate on N-linked sites, addition of carbohydrate to serine and threonine residues, sulfation of tyrosine residues, and cleavage of the protein to the mature heavy and light chains.

bonding pairs in factor VIII resulted in impaired secretion while elimination of the disulfide loop pairing residues 1899 to 1903 in factor VIII resulted in improved secretion [12].

Asparagine- and serine/threonine-linked glycosylation

Addition of N-linked oligosaccharides to many glycoproteins is an obligatory event for the folding and assembly of newly synthesized polypeptides. The presence of oligosaccharides is often required for the efficient transport of individual glycoproteins through the secretory pathway [13,14]. In addition, N-linked glycosylation frequently affects the plasma half-life and biologic activity of glycoproteins. The consensus site for N-linked glycosylation is Asn–Xxx–Ser/Thr, where Xxx may be any amino acid except for proline. The utilization of a particular consensus site for N-linked oligosaccharide attachment is determined by the structure of the growing polypeptide. As a consequence, proteins expressed in heterologous cells most frequently exhibit occupancy of N-linked sites very similar to that of the native polypeptide.

After addition of the high-mannose-containing oligosaccharide core structure (composed of glucose$_3$-mannose$_9$-N-acetylglucosamine$_2$) to consensus asparagine residues, trimming begins with the removal of the three terminal glucose residues that is mediated by the action of glucosidases I and II. Glucosidase I removes the terminal α_{1-3} glucose and glucosidase II subsequently removes the two α_{1-2} glucose residues. Glucose trimming is required for binding to the protein chaperones calnexin (CNX) and calreticulin (CRT) within the lumen of the ER. Prolonged association with CNX and/or CRT is observed when proteins are unfolded, misfolded, or unable to oligomerize. CNX and CRT bind most avidly to monoglucosylated forms of the N-linked core structure. Removal of the third glucose from the oligosaccharide core structure correlates with release from CNX and CRT and transport to the Golgi apparatus. The selectivity in binding of unfolded glycoproteins to CRT and CNX is mediated by reglucosylation of the deglucosylated N-linked oligosaccharide. This reglucosylation activity is performed by a UDP-glucose:glycoprotein glucosyltransferase (UGT). Only unfolded, mutant, or unassembled proteins are subject to reglucosylation. Reglucosylated proteins rebind CNX and/or CRT and, in this manner, unfolded proteins are retained in the ER through a cycle on CNX–CRT interaction, glucosidase II activity, and UGT activity. Subsequent to glucose trimming in the ER, at least one α_{1-2}-linked mannose is removed by an ER α_{1-2}-mannosidase prior to transport out of the ER. This system acts as a quality control mechanism allowing only properly folded factor V or factor VIII to be translocated out of the ER for secretion while sequestering or sending misfolded proteins to the proteasome through ER associates degradation pathways.

Upon transit through the Golgi apparatus, a series of additional carbohydrate modifications occur that are separated spatially and temporally and involve the removal of mannose residues by Golgi mannosidases I and II and the addition of N-acetylglucosamine, fucose, galactose, and sialic acid residues. These reactions occur by specific glycosyltransferases that modify the high-mannose carbohydrate to complex forms. Also within the Golgi apparatus, O-linked oligosaccharides are attached to the hydroxyl of serine or threonine residues through an O-glycosidic bond to N-acetylgalactosamine. Serine and threonine residues subject to glycosylation are frequently clustered together and contain an increased frequency of proline residues in the region, especially at positions −1 and +3, relative to the glycosylated residue. Galactose, fucose, and sialic acid are frequently attached to the serine/threonine-linked N-acetylgalactosamine. O-glycosylation occurs in the Golgi complex concomitant with complex processing of N-linked oligosaccharides.

Factor V and factor VIII contain a large number of N-linked oligosaccharides. Comparison of the N-linked oligosaccharides present on recombinant factor VIII expressed in mammalian cells to human plasma-derived factor VIII indicated that both proteins display similar occupancy and complexity

at the N-linked sites [6]. However, a detailed analysis demonstrated that differences in the microheterogeneity of oligosaccharides present on human plasma-derived factor VIII and recombinant factor VIII produced in baby hamster kidney cells do exist [15]. The light chains of factor VIIIa and factor Va migrate as doublets upon SDS-PAGE because of differences in the complexity of N-linked oligosaccharides present on the light chain [16]. The difference in complexity of the N-linked sugars on the light chain does not affect factor VIII activity. The majority of N-linked oligosaccharides within factor VIII and factor V occur within the B domain. Recent studies indicate that the N-linked oligosaccharides within the factor V and factor VIII B domains may be important to interact with the protein chaperone complex LMAN1–MCFD2 for facilitated transport from the ER to the Golgi compartment [17,18] (Figure 2.2). Mutations in either of the subunits of this heterodimeric complex cause combined deficiency of coagulation factors V and VIII [19,20]. MCFD2 appears to recruit factor V and VIII to the LMAN1 cargo complex and LMAN1 acts to recycle MCFD2 back to the ER. In the absence of LMAN1, MCFD2 is secreted from the cell [21]. In patients with combined factor V and VIII-deficiency plasma this results in lower circulating levels of factors V and VIII for patients with MCFD2 mutations compared with those with LMAN1 mutations [22].

Detailed analysis of recombinant factor VIII demonstrated that 3% of the total sugar chains contain a Galα_{1-3}Gal group on some of the outer chains of the bi, tri, and tetra-antennary complex-type sugar chains that is absent on factor VIII derived from human plasma. This structure was present in Kogenate® (prepared from baby hamster kidney cells) and not in Recombinate® [prepared from Chinese hamster ovary (CHO) cells] [15]. The α_{1-3}-galactosyltransferase that produces this structure is expressed in most nonprimate mammalian cells, and primates frequently develop antibodies to this structure. Approximately 1% of immunoglobulin in human plasma is directed toward this moiety, so it is expected that antibodies should be detected. A limited clinical trial did not detect any difference in the efficacy and/or half-life of factor VIII that contains the Galα_{1-3}Gal group. Therefore, there is no evidence of detrimental effects of this structure present on recombinant factor VIII.

Chaperone-assisted factor VIII folding

Factor VIII protein is secreted at markedly lower levels than similar proteins including factor V [23]. Secretion of factors V and VIII in the proper tertiary and quaternary structure requires considerable chaperone assistance in the ER. It has been established that folding of factor VIII is considerably more onerous than even the homologous protein factor V and this accounts for the major difference in secretion [16]. Misfolded, unfolded, or defective proteins are refolded, sequestered in the ER or degraded through ER-associated degradation (ERAD) by retrotranslocation into the cytosol and degradation by the 26S proteasome [24–27]. In addition to the calnexin/calreticulin system that acts in a "quality control" capacity, the ER has a complex tripartite system to match protein-folding capacity to protein load, termed the unfolded protein response (UPR) [28]. In addition to detection of unfolded proteins in the ER lumen, reactive oxygen species induced by factor VIII expression act a signal to activate the UPR [29]. These reactive oxygen species also impair protein folding and antioxidant treatment improved factor VIII secretion [29]. This pathway acts to decrease general protein translation, increase protein-folding chaperones, and increase degradation of misfolded proteins in order to decrease the protein-folding stress on the ER. The IRE/XBP-1 arm of the UPR is largely responsible for increasing chaperone-assisted protein-folding capacity through spliced XBP-1 mediated transcription of chaperones. Overexpression of spliced XBP-1 with factor VIII resulted in increased BiP/GRP78, an ER lumenal chaperone protein of the heat shock protein 70 (hsp70) family. As factor VIII is translated and translocated into the ER, BiP interacts with factor VIII transiently and retains factor VIII within the ER lumen. Expression of factor VIII induces transcription of the BiP gene [30] and the level of BiP inversely correlates with the factor VIII secretion efficiency. Overexpressed BiP complexes with factor VIII in the ER and results in decreased factor VIII secretion [31]. Site-directed mutation of F309S of factor impairs interaction with BiP and improves factor VIII secretion efficiency [32,33]. Dissociation of BiP from wild-type factor VIII requires much more ATP than dissociation from the F309S mutant [32,34].

Tyrosine sulfation

Sulfate addition to tyrosine as an O^4-sulfate ester is a common post-translational modification of secretory proteins that occurs in the trans-Golgi apparatus and is mediated by tyrosylprotein sulfotransferase that utilizes the activated sulfate donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS). This modification occurs on many secretory proteins including a number of proteins that interact with thrombin, such as hirudin, fibrinogen, heparin cofactor II, α_2-antiplasmin, vitronectin, and bovine factor X. In addition, both factor V and factor VIII contain multiple sites of tyrosine sulfation [35–37]. Tyrosine sulfation can modulate the biologic activity, binding affinities, and secretion of specific proteins. For example, tyrosine sulfation at the carboxy-terminus of hirudin increases its binding affinity to the anion-binding exosite of thrombin [38].

Recombinant factor VIII contains six sites of tyrosine sulfation at residues 346, 718, 719, 723, 1664, and 1680 [35]. All sites are sulfated to near completion, so it does not appear that this modification is inefficient in CHO cells. Site-directed mutagenesis was used to change individual or multiple tyrosine residues to the conserved residue phenylalanine in order to identify their role in factor VIII function. Tyrosine sulfation at all six sites was required for full factor VIII activity. In addition, mutagenesis of Tyr1680 to Phe demonstrated that

sulfation at that residue was required for high-affinity interaction with VWF [37,39]. In the absence of tyrosine sulfation at 1680 in factor VIII, the affinity for VWF was reduced by fivefold. In contrast, mutation at residue Tyr1664 did not affect VWF interaction. The significance of the Tyr1680 sulfation *in vivo* is made evident by the presence of a Tyr1680 to Phe mutation that causes a moderate hemophilia A, likely owing to reduced interaction with VWF and decreased plasma half-life [40]. The other sites of tyrosine sulfation within factor VIII affect the rate of cleavage by thrombin at the adjacent thrombin cleavage site. It was suggested that thrombin selectively utilizes the tyrosine sulfate residues adjacent to cleavage sites in factors V and VIII to facilitate interaction and/or cleavage.

Phosphorylation of serine and threonine residues

Phosphate has been observed in factor V and factor VIII, although its significance remains unknown. Exposure of factors V and VIII to activated platelets results in phosphorylation of serine residues in factor V and primarily threonine residues in factor VIII [41]. Phosphorylation can occur within the heavy chain of factor Va and both the heavy chain and light chains of factor VIII, possibly within the acid-rich regions. Phosphorylation of factor VIII by casein kinase II is thought to occur within the acidic regions 337 through 372 and 1649 through 1689. Although the kinase responsible for the phosphorylation remains unknown, it may be related to casein kinase II [42]. Partially phosphorylated factor Va was shown to be more sensitive to activated protein C (aPC) inactivation, suggesting that phosphorylation of these cofactors may downregulate their activity.

Proteolytic processing

Factor VIII proteolytic processing within the B domain after arginine residues 1313 and 1648 can saturate the proteolytic machinery of the cell. Both arginine residues at 1313 and 1648 have consensus sites for furin cleavage. In this case, secretion of heavy chains that extend to residue 1648 and secretion of light chains that extend to 1313 can be detected. In addition, some single-chain factor VIII is detected in conditioned medium from transfected mammalian cells and in heparin-treated human plasma [6,43]. However, all analyses to date indicate that these partially processed products of factor VIII have identical activity to fully processed factor VIII. For example, double mutation of Arg1313Ile and Arg1648Ile yields a single-chain factor VIII molecule with functional activity similar to wild-type factor VIII [16].

Summary

Eukaryotic cells contain an extensive machinery to modify polypeptides that transit the secretory compartment. In the case of coagulation factors VIII, a large number of post-translational modifications occur; many are required for secretion of the polypeptide and others are required for functional activity of the polypeptide. Proper synthesis and secretion of factor VIII requires that the primary translation product is modified by signal peptide cleavage and core high-mannose oligosaccharide addition upon translocation into the lumen of the ER. Within the ER, factor VIII requires trimming of glucose residues on the core N-linked glycols for transport to the Golgi compartment. In the Golgi compartment additional modifications occur, which include: (i) tyrosine sulfation of six residues that are required for efficient activation by thrombin and for high-affinity VWF interaction; (ii) extensive addition of oligosaccharides to many serine/threonine residues within the B domain; (iii) complex modification of N-linked glycols; and (iv) cleavage of single-chain factor VIII to its heavy- and light-chain species. To date, there do not appear to be any specific post-translational modifications that significantly limit secretion and/or functional activity of factor VIII. Further studies are required to elucidate the effect of factor VIII expression in different cell types in order to identify the importance that subtle differences in post-translational modifications may have on their secretion, *in vivo* half-life, and function. These considerations will be important when considering different cells and tissues as targets for gene therapy.

Factor IX

Factor IX and hemophilia B

Hemophilia B is caused by mutations in the *F9* gene leading to deficient or defective factor IX in its role as a serine protease in the intrinsic coagulation cascade. These mutations result in slow clot formation and prolonged bleeding. Hemophilia B was recognized as a clinical entity distinct from hemophilia A when it was noted that mixing plasma from patients with one hemophilia corrected the prolonged clotting times from patients with the other hemophilia [44,45]. The primary structure of human factor IX was determined from affinity-purified factor IX from plasma and the cDNA sequence was cloned using oligonucleotides derived from the bovine factor IX amino acid sequence [46–48]. This work led to the development of recombinant factor IX produced in CHO cells for clinical use in patients with hemophilia B [49,50]. Prior to being secreted from hepatocytes, factor IX undergoes γ-carboxylation, O- and N-linked glycosylation, phosphorylation, sulfation, disulfide bond formation, and β-hydroxylation, as well as cleavage of the signal peptide and propeptide. Gene therapy trials in hemophilia B have shown safety, but, to date, little efficacy [51]. Improved understanding of the mechanisms of factor IX processing and secretion has important implications for both production of recombinant factor IX and future gene therapy trials in hemophilia B.

The domain structures of the vitamin K-dependent coagulation factors factor VII, factor IX, factor X, prothrombin,

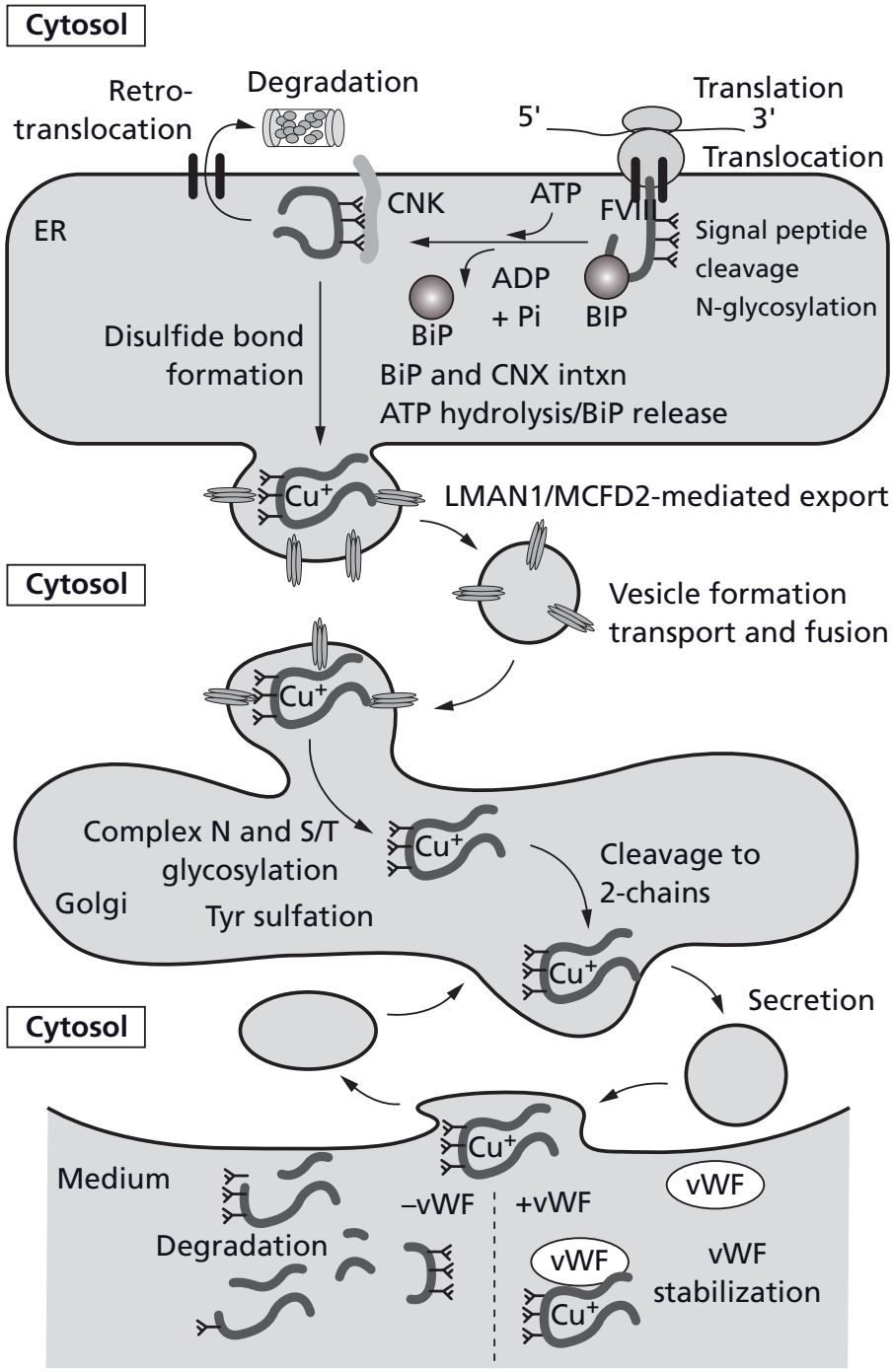

Figure 2.3 Domain structure and processing of factor IX. Factor IX is composed of a signal peptide, propeptide, γ-carboxyglutamic acid domain (GLA), epidermal growth factor (EGF)-like domains, activation peptide, and serine protease catalytic domain. Short arrows represent intracellular processing sites that cleave away the signal peptide and the propeptide. The long arrows represent the cleavages required for activation by factor VIIa/tissue factor (VIIa/TF) or factor XIa. The 35 amino acid activation peptide is indicated. γ represents γ-carboxyglutamic acid and β represents β-hydroxyaspartic acid. The 330–338 loop that interacts with factor VIII is shown by a dotted line. Also indicated are sites of addition of asparagine-linked oligosaccharides (N), serine or threonine-linked oligosaccharides (S and T, respectively), tyrosine sulfation (Y-S), and serine phosphorylation (S-P).

protein C, and protein S deduced from their cDNA sequences demonstrate that they contain common structural features (Figure 2.3) [52]. All contain a signal peptide that is required for translocation into the lumen of the ER. This is followed by a propeptide that directs vitamin K-dependent γ-carboxylation of the mature polypeptide. Upon transit through the trans-Golgi apparatus, the propeptide is cleaved away. The amino-terminus of the mature protein contains a γ-carboxy glutamic acid-rich region (Gla) that includes a short α-helical stack of aromatic amino acids. Then there are two epidermal growth factor (EGF)-like domains. In factor IX, protein C, and factor X, the amino-terminal EGF domain contains β-hydroxyaspartic acid (Hya) at homologous locations. The next region is the activation peptide (12–52 residues) that is glycosylated on asparagine residues and is released by specific proteolysis accompanying activation. The remainder of the vitamin K-dependent protease comprises the serine protease catalytic triad that is absent in protein S.

Disulfide bond formation

The vitamin K-dependent coagulation factors, exemplified by factor IX, have conserved disulfide bonds. Generally, three disulfide bonds occur within each EGF domain, and several disulfide bonds occur within the serine protease catalytic domain. In addition, factor IX has a disulfide bond that connects the amino-terminal half with the carboxy-terminal half of the protein so that after activation the two portions of the molecule do not dissociate. In factor IX, cysteine residues at 18 and 23 within the Gla domain form a small essential disulfide loop where mutations at either cysteine residue results in severe hemophilia B [53].

Asparagine- and serine/threonine-linked glycosylation

With the development of recombinant factor IX produced in CHO cells for treatment of hemophilia B, a detailed characterization and comparison of the carbohydrate structures was performed between plasma-derived and recombinant-derived factor IX [54]. In both plasma- and recombinant- derived factor IX, Asn157 and Asn167 within the activation peptide are fully occupied with complex-type N-glycans [55]. Recombinant factor IX contains tetra-antennary, tetrasialylated, and core fucosylated glycans at both sites. Plasma-derived factor IX contains bi-, tri-, and tetra-antennary, sialylated glycans, with and without fucose. Both molecules have a range of minor structures; however, the glycans present on plasma-derived factor IX are considerably more heterogeneous and diverse. The diversity may be a consequence of the plasma pool.

Both plasma- and recombinant-derived factor IX contain a number of O-linked oligosaccharides. In the first factor IX EGF domain, serine residues 53 and 61 are uniformly O-glycosylated. The EGF1 domain in both recombinant and plasma-derived factor IX contains nonclassical O-linked glycans at Ser53 and Ser61. Ser53 contains Xyl–Xyl–Glc–Ser and Ser61 contains the tetrasaccharide with a terminal sialic acid (NeuAc), NeuAc–Gal–GlcNac–Fuc–Ser61 [56–58]. This indicates that CHO cells (the cells used as a host to produce recombinant factor IX) have the enzymatic machinery to produce the structures present on plasma-derived factor IX that is synthesized in human hepatocytes and that it is not saturated at high expression levels. The carbohydrate structure at Ser61 in factor IX contains fucose-linked tetrasaccharide with a terminal sialic acid. Ser61 within the first EGF domain of factor IX has the consensus sequence (C–X–X–G–G–T/S–C) for fucosyl modification of O-linked sugars and is also found in factor VII, but not in factor X. However, a crystal structure of factor IX demonstrated that both these O-linked modifications reside on the face of the EGF domain that apparently does not interact with other components of the factor Xase complex [59]. In addition to the serine-linked oligosaccharide addition in the first EGF domain, both plasma-derived and recombinant-derived factor IX molecules are partially occupied by O-linked glycans at residues Thr159, Thr169, Thr172, and Thr179, as well as as-yet unidentified additional sites [55]. The function of these O-linked glycans remains unknown.

γ-Carboxylation of glutamic acid residues

The vitamin K-dependent coagulation factors contain the post-translationally modified amino acid γ-carboxy-glutamic acid (Gla). The Gla residues are essential for these proteins to attain a calcium-dependent conformation and for their ability to bind to phospholipid surfaces, an essential interaction for their function. The precursor of the vitamin K-dependent coagulation factors contains a propeptide that directs γ-carboxylation of up to 12 glutamic acid residues at the amino-terminus of the mature protein, all of which is completed prior to translocation out of the ER [60–62]. The propeptides (residues –18 to –1 in factor IX) of these factors share amino acid similarity by conservation of the γ-carboxylase recognition site and the site for cleavage of the propeptide.

The residues that are carboxylated in factor IX are glutamic acid residues 7, 8, 15, 17, 20, 21, 26, 27, 30, 33, 36, and 40. Mutations at residues 6, 7, 17, 21, 27, 30, or 33 result in moderate to severe hemophilia B, indicating their functional importance. High-level expression of the vitamin K-dependent plasma proteins in transfected mammalian cells is limited by the ability of the mammalian host cell to efficiently perform γ-carboxylation of amino-terminal glutamic acid residues and also to efficiently cleave the propeptide [49,63,64]. Analysis of factor IX expressed in CHO cells revealed that the protein had a much lower specific activity compared with the natural human plasma-derived protein. The reduced specific activity was attributed to both the limited ability of CHO cells to cleave the propeptide of factor IX as well to efficiently perform γ-carboxylation [49,64]. Generally, expression of factor IX at levels greater than 1 μg/10^6 cells/day saturates the activity for most cells studied [63]. Overexpression of the γ-carboxylase did not improve γ-carboxylation of factor IX when coexpressed in transfected mammalian cells [49]. These results suggest that the amount of carboxylase protein is not a limiting factor to direct vitamin K-dependent γ-carboxylation *in vivo*. Several possibilities exist for the inability of the overexpressed γ-carboxylase to improve factor IX carboxylation *in vivo*. First, the overexpressed γ-carboxylase may be mislocalized within the secretory pathway. It is possible that another protein, such as a protein chaperone, may be required to utilize a more complex substrate, such as factor IX, as opposed to a small peptide substrate. It is possible that another cofactor, possibly reduced vitamin K, is limiting for factor IX carboxylation *in vivo*. Further information on the mechanism of γ-carboxylation reaction *in vivo* is required in order to elucidate the rate-limiting step for γ-carboxylation *in vivo*.

Recombinant factor IX produced in CHO cells contains 11.8 Gla residues/mole of factor IX, compared with plasma-derived factor IX that contains 12 Gla residues/mole. The difference resides in the inefficient carboxylation of residues 36 and 40 within recombinant factor IX [65]. In contrast to the first 10 Gla residues in factor IX, glutamic acid residues 36 and 40 are not conserved in the other vitamin K-dependent coagulation factors. To date, no functional difference is observed between fully carboxylated factor IX and factor IX deficient in Gla at residues 36 and 40.

β-Hydroxylation of aspartic acid and asparagine

Blood coagulation factors IX and X, protein C, and protein S contain the modified amino acid erythro-β-hydroxyaspartic acid in the first EGF domain. In addition, one molecule of β-hydroxyasparagine is found in each of the three carboxy-terminal EGF domains in protein S. Hydroxylation of both aspartic acid and asparagine is catalyzed by aspartyl β-hydroxylase, requires 2-ketoglutarate and Fe^{2+}, and is inhibited by agents that inhibit 2-ketoglutarate-dependent dioxygenases. This enzyme recognizes a consensus sequence C–X–X–X–X–X–X–X–X–C in the β-sheet and C–X–D/N–X–X–X–X–Y/F–X in the antiparallel β-sheet [66]. β-hydroxylation is unnecessary for high-affinity calcium binding to the first EGF domain [67]. In addition, inhibition of β-hydroxylation of factor IX expressed in mammalian cells did not reduce functional activity in factor IX [68]. It is interesting that only 30% of plasma factor IX is modified by β-hydroxylation at Asp64 and this same amount of β-hydroxylation occurs in recombinant factor IX expressed at high levels in CHO cells [68].

Tyrosine sulfation

Plasma-derived and recombinant-derived factor IX are sulfated on Tyr155. Whereas plasma-derived factor IX is mostly sulfated, recombinant factor IX is approximately 15% sulfated [54,58]. This is one unusual example where a sulfated tyrosine occurs adjacent to an occupied N-linked glycosylation site (at asparagine residue 157). Plasma-derived factor IX and plasma-derived factor IX differ in their *in vivo* recovery, where the absolute recovery of plasma-derived factor IX is approximately 50% and the recovery of recombinant factor IX is approximately 30%. Studies suggest tyrosine sulfation on factor IX may be responsible for the difference in the recovery of these two sources of factor IX [58]. For example, infusion of recombinant factor IX enriched for full sulfation at Tyr155 demonstrated an equivalent recovery to plasma-derived factor IX (approximately 50%). Similarly, removal of the sulfate, as well as phosphate, from plasma-derived factor IX resulted in a molecule having a recovery similar to recombinant factor IX. Finally, administration of recombinant factor IX to hemophilia B dogs and isolation of the circulating factor IX yielded species that were enriched with tyrosine sulfate compared with the starting material. The sum of these observations suggests that tyrosine sulfation at 155 in factor IX can influence *in vivo* recovery.

Phosphorylation of serine and threonine residues

Phosphate has been observed in factor IX, although its significance remains unknown. Plasma-derived factor IX is fully phosphorylated at Ser158 whereas recombinant factor IX contains no phosphate at this position [58,69]. Factor IX produced in myotubes had considerably less phosphorylation at Ser158 compared with plasma-derived factor IX but maintained similar specific activity [70]. The presence or absence of phosphate or sulfate on factor IX has no effect on the *in vitro* clotting activity.

Proteolytic processing

The requirement for propeptide processing for factor IX function was first made apparent by identification mutations resulting in hemophilia B that prevent processing of the factor IX propeptide. Mutations of the Arg at the P1 or P4 positions inhibit propeptide cleavage and the resultant factor IX is secreted into the plasma, but is nonfunctional because of the presence of the propeptide [71,72]. This mutant is unable to bind phospholipid vesicles and may also display reduced γ-carboxylation of glutamic acid residues [72]. It is likely that the presence of the propeptide yields a molecule that is defective in phospholipid interaction as a result of an inability to undergo a calcium-dependent conformation in the Gla domain.

Characterization of the amino acid requirements around the propeptide cleavage site has identified that both the P1 and P4 arginine are important for efficient processing mediated by furin and PACE4 [73,74]. Overexpression of furin in transfected cells as well as in transgenic animals improves the processing ability to yield fully processed proteins [64]. Recombinant factor IX is produced by coexpression with furin/PACE to ensure complete processing of the propeptide.

Summary

Factor IX undergoes a remarkable number of varied post-translational modifications prior to secretion from the hepatocyte. Cotranslational translocation into the lumen of the ER occurs concomitantly with signal peptide cleavage and addition of core high-mannose oligosaccharides to the polypeptide is followed in the ER by glucose trimming of the N-linked oligosaccharide core structures, γ-carboxylation of 12 amino-terminal glutamic acid residues, and β-hydroxylation of a portion of molecules on residue Asp64 occurs. Upon transit into the Golgi compartment, additional modifications occur, which include (i) complex modification of N-linked oligosaccharides; (ii) tyrosine sulfation at Tyr155; (iii) serine/threonine glycosylation at residues Ser61 and Ser53, as well as several threonine residues within the activation peptide; and (iv)

cleavage of the propeptide. In addition, factor IX isolated from human plasma is phosphorylated at Ser158 within the activation peptide. A majority of the modifications within factor IX occur within the activation peptide and may regulate activation of factor IX. Appropriate γ-carboxylation and propeptide cleavage are essential for functional secretion and activity of secreted factor IX. Both of these activities are easily saturated upon expression of factor IX in heterologous cells. The large number of other modifications likely also affects factor IX activity by mechanisms that are not understood to date. Further studies are required to elucidate the effect of factor IX expression in different cell types in order to identify the importance that subtle differences in post-translational modifications may have on their secretion, *in vivo* half-life, and function. These considerations will be important when considering different cells and tissues as targets for gene therapy.

References

1 Toole JJ, Knopf JL, Wozney JM, *et al.* Molecular cloning of a cDNA encoding human antihaemophilic factor. *Nature* 1984; **312**: 342–7.

2 Bihoreau N, Pin S, de Kersabiec AM, Vidot F, Fontaine-Aupart MP. Copper-atom identification in the active and inactive forms of plasma-derived FVIII and recombinant FVIII-delta II. *Eur J Biochem* 1994; **222**: 41–8.

3 Tagliavacca L, Moon N, Dunham WR, Kaufman RJ. Identification and functional requirement of Cu(I) and its ligands within coagulation factor VIII. *J Biol Chem* 1997; **272**: 27428–34.

4 Ngo JC, Huang M, Roth DA, Furie BC, Furie B. Crystal structure of human factor VIII: implications for the formation of the factor IXa-factor VIIIa complex. *Structure* 2008; **16**: 597–606.

5 Shen BW, Spiegel PC, Chang CH, *et al.* The tertiary structure and domain organization of coagulation factor VIII. *Blood* 2008; **111**: 1240–7.

6 Kaufman RJ, Wasley LC, Dorner AJ. Synthesis, processing, and secretion of recombinant human factor VIII expressed in mammalian cells. *J Biol Chem* 1988; **263**: 6352–62.

7 Weiss HJ, Sussman, II, Hoyer LW. Stabilization of factor VIII in plasma by the von Willebrand factor. Studies on posttransfusion and dissociated factor VIII and in patients with von Willebrand's disease. *J Clin Invest* 1977; **60**: 390–404.

8 Eaton D, Rodriguez H, Vehar GA. Proteolytic processing of human factor VIII. Correlation of specific cleavages by thrombin, factor Xa, and activated protein C with activation and inactivation of factor VIII coagulant activity. *Biochemistry* 1986; **25**: 505–12.

9 Fay PJ, Haidaris PJ, Smudzin TM. Human factor VIIIa subunit structure. Reconstruction of factor VIIIa from the isolated A1/A3-C1-C2 dimer and A2 subunit. *J Biol Chem* 1991; **266**: 8957–62.

10 Lollar P, Parker CG, Kajenski PJ, Litwiller RD, Fass DN. Degradation of coagulation proteins by an enzyme from Malayan pit viper (*Akistrodon rhodostoma*) venom. *Biochemistry* 1987; **26**: 7627–36.

11 McMullen BA, Fujikawa K, Davie EW, Hedner U, Ezban M. Locations of disulfide bonds and free cysteines in the heavy and light chains of recombinant human factor VIII (antihemophilic factor A). *Protein Sci* 1995; **4**: 740–6.

12 Selvaraj SR, Kaufman RJ, Pipe SW. Incremental improvement in bioengineering of coagulation factor VIII for efficient expression: elimination of a dispensable disulfide loop enhances secretion. *Blood* (ASH Annual Meeting Abstracts) 2008; **112**: 3073.

13 Sousa MC, Ferrero-Garcia MA, Parodi AJ. Recognition of the oligosaccharide and protein moieties of glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase. *Biochemistry* 1992; **31**: 97–105.

14 Sousa M, Parodi AJ. The molecular basis for the recognition of misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase. *EMBO J* 1995; **14**: 4196–203.

15 Hironaka T, Furukawa K, Esmon PC, *et al.* Comparative study of the sugar chains of factor VIII purified from human plasma and from the culture media of recombinant baby hamster kidney cells. *J Biol Chem* 1992; **267**: 8012–20.

16 Pittman DD, Tomkinson KN, Kaufman RJ. Post-translational requirements for functional factor V and factor VIII secretion in mammalian cells. *J Biol Chem* 1994; **269**: 17329–37.

17 Moussalli M, Pipe SW, Hauri HP, Nichols WC, Ginsburg D, Kaufman RJ. Mannose-dependent endoplasmic reticulum (ER)-Golgi intermediate compartment-53-mediated ER to Golgi trafficking of coagulation factors V and VIII. *J Biol Chem* 1999; **274**: 32539–42.

18 Cunningham MA, Pipe SW, Zhang B, Hauri HP, Ginsburg D, Kaufman RJ. LMAN1 is a molecular chaperone for the secretion of coagulation factor VIII. *J Thromb Haemost* 2003; **1**: 2360–7.

19 Nichols WC, Seligsohn U, Zivelin A, *et al.* Mutations in the ER–Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of coagulation factors V and VIII. *Cell* 1998; **93**: 61–70.

20 Zhang B, Cunningham MA, Nichols WC, *et al.* Bleeding due to disruption of a cargo-specific ER-to-Golgi transport complex. *Nat Genet* 2003; **34**: 220–5.

21 Nyfeler B, Zhang B, Ginsburg D, Kaufman RJ, Hauri HP. Cargo selectivity of the ERGIC-53/MCFD2 transport receptor complex. *Traffic* 2006; **7**: 1473–81.

22 Zhang B, Spreafico M, Zheng C, *et al.* Genotype-phenotype correlation in combined deficiency of factor V and factor VIII. *Blood* 2008; **111**: 5592–600.

23 Lynch CM, Israel DI, Kaufman RJ, Miller AD. Sequences in the coding region of clotting factor VIII act as dominant inhibitors of RNA accumulation and protein production. *Hum Gene Ther* 1993; **4**: 259–72.

24 Eriksson KK, Vago R, Calanca V, Galli C, Paganetti P, Molinari M. EDEM contributes to maintenance of protein folding efficiency and secretory capacity. *J Biol Chem* 2004; **279**: 44600–5.

25 Molinari M, Calanca V, Galli C, Lucca P, Paganetti P. Role of EDEM in the release of misfolded glycoproteins from the calnexin cycle. *Science* 2003; **299**: 1397–400.

26 Oda Y, Hosokawa N, Wada I, Nagata K. EDEM as an acceptor of terminally misfolded glycoproteins released from calnexin. *Science* 2003; **299**: 1394–7.

27 Kaufman RJ. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. *Genes Dev* 1999; **13**: 1211–33.

28 Kaufman RJ. Orchestrating the unfolded protein response in health and disease. *J Clin Invest* 2002; **110**: 1389–98.

29 Malhotra JD, Miao H, Zhang K, *et al.* Antioxidants reduce endoplasmic reticulum stress and improve protein secretion. *Proc Natl Acad Sci USA* 2008; **105**: 18525–30.

30 Dorner AJ, Wasley LC, Kaufman RJ. Increased synthesis of secreted proteins induces expression of glucose-regulated proteins in butyrate-treated Chinese hamster ovary cells. *J Biol Chem* 1989; **264**: 20602–7.
31 Morris JA, Dorner AJ, Edwards CA, Hendershot LM, Kaufman RJ. Immunoglobulin binding protein (BiP) function is required to protect cells from endoplasmic reticulum stress but is not required for the secretion of selective proteins. *J Biol Chem* 1997; **272**: 4327–34.
32 Swaroop M, Moussalli M, Pipe SW, Kaufman RJ. Mutagenesis of a potential immunoglobulin-binding protein-binding site enhances secretion of coagulation factor VIII. *J Biol Chem* 1997; **272**: 24121–4.
33 Tagliavacca L, Wang Q, Kaufman RJ. ATP-dependent dissociation of non-disulfide-linked aggregates of coagulation factor VIII is a rate-limiting step for secretion. *Biochemistry* 2000; **39**: 1973–81.
34 Dorner AJ, Wasley LC, Kaufman RJ. Protein dissociation from GRP78 and secretion are blocked by depletion of cellular ATP levels. *Proc Natl Acad Sci USA* 1990; **87**: 7429–32.
35 Pittman DD, Wang JH, Kaufman RJ. Identification and functional importance of tyrosine sulfate residues within recombinant factor VIII. *Biochemistry* 1992; **31**: 3315–25.
36 Pittman DD, Tomkinson KN, Michnick D, Selighsohn U, Kaufman RJ. Posttranslational sulfation of factor V is required for efficient thrombin cleavage and activation and for full procoagulant activity. *Biochemistry* 1994; **33**: 6952–9.
37 Michnick DA, Pittman DD, Wise RJ, Kaufman RJ. Identification of individual tyrosine sulfation sites within factor VIII required for optimal activity and efficient thrombin cleavage. *J Biol Chem* 1994; **269**: 20095–102.
38 Rydel TJ, Ravichandran KG, Tulinsky A, *et al.* The structure of a complex of recombinant hirudin and human alpha-thrombin. *Science* 1990; **249**: 277–80.
39 Leyte A, van Schijndel HB, Niehrs C, *et al.* Sulfation of Tyr1680 of human blood coagulation factor VIII is essential for the interaction of factor VIII with von Willebrand factor. *J Biol Chem* 1991; **266**: 740–6.
40 Higuchi M, Wong C, Kochhan L, *et al.* Characterization of mutations in the factor VIII gene by direct sequencing of amplified genomic DNA. *Genomics* 1990; **6**: 65–71.
41 Rand MD, Kalafatis M, Mann KG. Platelet coagulation factor Va: the major secretory platelet phosphoprotein. *Blood* 1994; **83**: 2180–90.
42 Kalafatis M, Rand MD, Jenny RJ, Ehrlich YH, Mann KG. Phosphorylation of factor Va and factor VIIIa by activated platelets. *Blood* 1993; **81**: 704–19.
43 Ganz PR, Tackaberry ES, Palmer DS, Rock G. Human factor VIII from heparinized plasma. Purification and characterization of a single-chain form. *Eur J Biochem* 1988; **170**: 521–8.
44 Aggeler PM, White SG, Glendening MB, Page EW, Leake TB, Bates G. Plasma thromboplastin component (PTC) deficiency; a new disease resembling hemophilia. *Proc Soc Exp Biol Med* 1952; **79**: 692–4.
45 Schulman E, Smith CH. Hemorrhagic disease in an infant due to deficiency of a previously undescribed coagulation factor. *AMA Am J Dis Child* 1952; **84**: 758–60.
46 Andersson LO, Borg H, Miller-Andersson M. Purification and characterization of human factor IX. *Thromb Res* 1975; **7**: 451–9.
47 Choo KH, Gould KG, Rees DJ, Brownlee GG. Molecular cloning of the gene for human anti-haemophilic factor IX. *Nature* 1982; **299**: 178–80.
48 Kurachi K, Davie EW. Isolation and characterization of a cDNA coding for human factor IX. *Proc Natl Acad Sci USA* 1982; **79**: 6461–4.
49 Kaufman RJ, Wasley LC, Furie BC, Furie B, Shoemaker CB. Expression, purification, and characterization of recombinant gamma-carboxylated factor IX synthesized in Chinese hamster ovary cells. *J Biol Chem* 1986; **261**: 9622–8.
50 Roth DA, Kessler CM, Pasi KJ, *et al.* Human recombinant factor IX: safety and efficacy studies in hemophilia B patients previously treated with plasma-derived factor IX concentrates. *Blood* 2001; **98**: 3600–6.
51 Murphy SL, High KA. Gene therapy for haemophilia. *Br J Haematol* 2008; **140**: 479–87.
52 Davie EW. Biochemical and molecular aspects of the coagulation cascade. *Thromb Haemost* 1995; **74**: 1–6.
53 Wojcik EG, van den Berg M, van der Linden IK, Poort SR, Cupers R, Bertina RM. Factor IX Zutphen: a Cys18–>Arg mutation results in formation of a heterodimer with alpha 1-microglobulin and the inability to form a calcium-induced conformation. *Biochem J* 1995; **311**: 753–9.
54 White GC, 2nd, Beebe A, Nielsen B. Recombinant factor IX. *Thromb Haemost* 1997; **78**: 261–5.
55 Bond MD, Huberty MC, Jankowski MA. Identification of O-glycosylation, sulfation and phosphorylation sites in the activation peptide of human plasma factor IX. *Blood* (ASH Annual Meeting Abstracts) 1994; **84**: 531a.
56 Hase S, Nishimura H, Kawabata S, Iwanaga S, Ikenaka T. The structure of (xylose)2glucose-O-serine 53 found in the first epidermal growth factor-like domain of bovine blood clotting factor IX. *J Biol Chem* 1990; **265**: 1858–61.
57 Harris RJ, van Halbeek H, Glushka J, *et al.* Identification and structural analysis of the tetrasaccharide NeuAc alpha(2–>6)Gal beta(1–>4)GlcNAc beta(1–>3)Fuc alpha 1–>O-linked to serine 61 of human factor IX. *Biochemistry* 1993; **32**: 6539–47.
58 Bond MD, Jankowski MA, Huberty MC. Structural analysis of recombinant human factor IX. *Blood* 1994; **84**: 194a.
59 Brandstetter H, Bauer M, Huber R, Lollar P, Bode W. X-ray structure of clotting factor IXa: active site and module structure related to Xase activity and hemophilia B. *Proc Natl Acad Sci USA* 1995; **92**: 9796–800.
60 Bristol JA, Ratcliffe JV, Roth DA, Jacobs MA, Furie BC, Furie B. Biosynthesis of prothrombin: intracellular localization of the vitamin K-dependent carboxylase and the sites of gamma-carboxylation. *Blood* 1996; **88**: 2585–93.
61 Morris DP, Stevens RD, Wright DJ, Stafford DW. Processive post-translational modification. Vitamin K-dependent carboxylation of a peptide substrate. *J Biol Chem* 1995; **270**: 30491–8.
62 Fryklund L, Borg H, Andersson LO. Amino-terminal sequence of human factor IX: presence of gamma-carboxyl glutamic acid residues. *FEBS Lett* 1976; **65**: 187–9.
63 Wasley LC, Rehemtulla A, Bristol JA, Kaufman RJ. PACE/furin can process the vitamin K-dependent pro-factor IX precursor within the secretory pathway. *J Biol Chem* 1993; **268**: 8458–65.
64 Rehemtulla A, Roth DA, Wasley LC, *et al. In vitro* and *in vivo* functional characterization of bovine vitamin K-dependent gamma-carboxylase expressed in Chinese hamster ovary cells. *Proc Natl Acad Sci USA* 1993; **90**: 4611–5.
65 Gillis S, Furie BC, Furie B, *et al.* gamma-Carboxyglutamic acids 36 and 40 do not contribute to human factor IX function. *Protein Sci* 1997; **6**: 185–96.
66 Stenflo J, Stenberg Y, Muranyi A. Calcium-binding EGF-like modules in coagulation proteinases: function of the calcium ion in module interactions. *Biochim Biophys Acta* 2000; **1477**: 51–63.

67 Sunnerhagen MS, Persson E, Dahlqvist I, *et al*. The effect of aspartate hydroxylation on calcium binding to epidermal growth factor-like modules in coagulation factors IX and X. *J Biol Chem* 1993; **268**: 23339–44.
68 Derian CK, VanDusen W, Przysiecki CT, *et al*. Inhibitors of 2-ketoglutarate-dependent dioxygenases block aspartyl beta-hydroxylation of recombinant human factor IX in several mammalian expression systems. *J Biol Chem* 1989; **264**: 6615–8.
69 Atoda H, Yokota E, Morita T. Characterization of a monoclonal antibody B1 that recognizes phosphorylated Ser-158 in the activation peptide region of human coagulation factor IX. *J Biol Chem* 2006; **281**: 9314–20.
70 Arruda VR, Hagstrom JN, Deitch J, *et al*. Posttranslational modifications of recombinant myotube-synthesized human factor IX. *Blood* 2001; **97**: 130–8.
71 Bentley AK, Rees DJ, Rizza C, Brownlee GG. Defective propeptide processing of blood clotting factor IX caused by mutation of arginine to glutamine at position –4. *Cell* 1986; **45**: 343–8.
72 Diuguid DL, Rabiet MJ, Furie BC, Liebman HA, Furie B. Molecular basis of hemophilia B: a defective enzyme due to an unprocessed propeptide is caused by a point mutation in the factor IX precursor. *Proc Natl Acad Sci USA* 1986; **83**: 5803–7.
73 Rehemtulla A, Kaufman RJ. Preferred sequence requirements for cleavage of pro-von Willebrand factor by propeptide-processing enzymes. *Blood* 1992; **79**: 2349–55.
74 Rehemtulla A, Barr PJ, Rhodes CJ, Kaufman RJ. PACE4 is a member of the mammalian propeptidase family that has overlapping but not identical substrate specificity to PACE. *Biochemistry* 1993; **32**: 11586–90.

3 Molecular basis of hemophilia A

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Introduction

It is now over 25 years since the factor VIII (FVIII) protein was first purified [1], leading to the subsequent cloning of the gene [2]. Detection of causative mutations in the FVIII gene (*F8*) was initially slow and laborious, but recent years have seen great advances in the technology for detection of variations in *F8*. These, coupled with the recently published crystal structure of FVIII [3,4], have paved the way for a much greater understanding of the relationship between FVIII structure and the function of the cofactor in coagulation. There are still many questions to answer as to how FVIIIa (the thrombin-activated form of FVIII) interacts with phospholipid membranes and promotes the activity of activated factor IX (FIXa) so enormously in the activation of factor X (FX). Comprehensive mutation detection is often now performed by polymerase chain reaction (PCR) and direct sequencing of all exons. Because of the size and complexity of the *F8* gene this is still a considerable undertaking in many centers. FVIII function is briefly described below; however, for details of current thinking on the functional aspects of FVIII, readers may look to Chapter 4 of this volume.

Structure and function of the factor VIII gene (*F8*) and protein

F8 gene

The human *F8* gene was cloned between 1982 and 1984 simultaneously by two groups [2,5]. At the time, the gene was the largest described (186 kb), and is still one of the largest. Mapping positioned the *F8* gene in the most distal band (Xq28) of the long arm of the X chromosome. As shown in Figure 3.1, the gene contains 26 exons, 24 of which vary in length from 69 to 262 base pairs (bp); the remaining much larger exons, 14 and 26, contain 3106 and 1958 bp, respectively. However, the large majority of exon 26 is 3′ untranslated sequence, so that exon 14 bears by far the largest exonic coding sequence, largely that of the B domain (see below). The spliced FVIII mRNA is approximately 9 kb in length and predicts a precursor protein of 2351 amino acids.

Production of factor VIII protein

After removal of the 19 peptide secretory leader sequence, FVIII has a mature sequence of 2332 amino acids with the domain structure A1–*a1*–A2–*a2*–B–*a3*–A3–C1–C2 [6]. Figure 3.1 shows the relationship between the domains and the cDNA exons. FVIII circulates in plasma complexed noncovalently with von Willebrand factor (VWF), which acts as a plasma carrier, apparently protecting it from proteolysis and rapid clearance.

The domain structure of FVIII is very similar to that of coagulation factor V (FV) [7], although the B domains are unrelated in sequence and FV lacks the short acidic *a1*, *a2*, and *a3* sequences. The A domains of FVIII and FV are homologous to ceruloplasmin and hephaestin (which share the domain structure A1–A2–A3), and homology of the FVIII and FV C domains has been noted with slime mold discoidin I [6], milk-fat globule membrane protein [8], and a receptor tyrosine kinase found in breast carcinoma cells [9]. There is no significant homology between the B domain of FVIII and any other protein sequence in the human genome; however, the B domains of FVIII and FV share the characteristic that they are large and very heavily N-glycosylated, a factor that may be crucial in their intracellular folding and processing (see Chapter 2).

Factor VIII is highly sensitive to proteolytic processing after secretion, and only a small fraction of circulating FVIII is in the single-chain form: the majority consists of heavy chains of variable length (consisting of the A1 and A2 domains together with variable lengths of B domain) linked noncovalently to light chains consisting of the A3, C1, and C2 domains. Expression of active recombinant FVIII lacking the entire length of the B domain has confirmed that this domain is unnecessary for activation of the protein or procoagulant function [10]: a cleavage after R759 (probably by thrombin) during coagulation serves to remove it. In contrast, the FV B domain is important for normal activation of the cofactor.

The function of FVIII is to accelerate the activation of FX by FIXa on a suitable phospholipid surface, thus amplifying the clotting stimulus many-fold, and specific proteolytic cleavages between the domains both activate and inactivate the cofactor (see Chapter 4). It is clear that the active form (FVIIIa)

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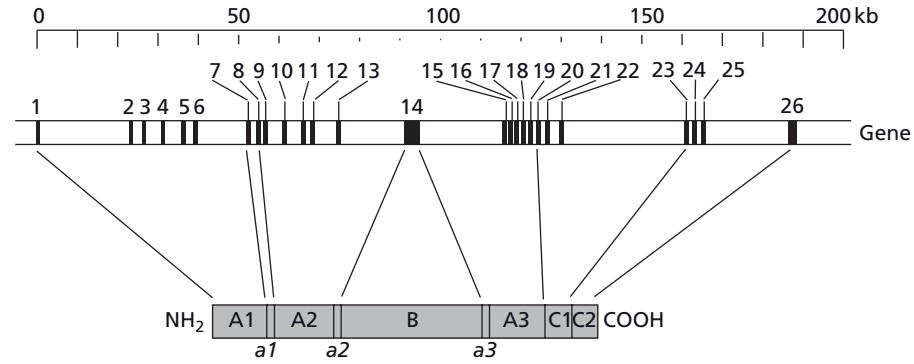

Figure 3.1 Linear representation of the *F8* gene showing (above) the 26 exons and (below) the domain organization of the protein based on amino acid homology comparisons.

consists of a heterotrimer of an A1–*a1* chain linked by a metal cation-dependent bond to the A3–C1–C2 chain; with the A2–*a2* chain held by electrostatic association between the A1 and A2 domains. Inactivation and loss of procoagulant function occurs through either spontaneous dissociation of A2–*a2* or proteolysis of FVIIIa by activated protein C (aPC).

Until recently, three-dimensional structural analysis of FVIII was based upon homology models using the separate structures of similar domains. In 2008, two groups independently solved the structure of B-domain-deleted FVIII by X-ray crystallography at intermediate resolution [3,4]. This demonstrated that the heterotrimer consists of the five expected globular domains with a metal ion bridge between the A1 and A3 domains. The C1, C2, and A3 domains contain phospholipid-binding regions that serve to anchor the cofactor on the negatively charged surface of activated platelets that form the platform for coagulation amplification. The crystal structure has been used to produce a putative model of the "tenase" complex in which FVIIIa and FIXa interact over an extensive surface including two high-affinity binding sites for FIXa in A2 and one in A3. The main advantage of the crystal structure is that it allows mapping of naturally occurring hemophilia A mutants with accurate prediction of the deleterious effects of mutating specific residues. Although this clearly represents a major advance, there are still a number of questions that remain to be answered regarding the purpose of the B domain, the mechanisms by which FVIIIa accelerates the catalytic function of FIXa and the interaction between FVIII and VWF that serves to stabilize FVIII in the circulation.

F8 gene defects found in hemophilia A

F8 gene defects associated with hemophilia A may be divided for convenience into several categories: (i) gross gene rearrangements; (ii) insertions or deletions of genetic sequence of a size varying from one base pair up to the entire gene; and (iii) single DNA base substitutions resulting in either amino acid replacement ("missense"), premature peptide chain termination ("nonsense" or stop mutations), or mRNA splicing defects. These classes are described briefly below.

At its last update in 2007, the international online hemophilia A mutation database (http://europium.csc.mrc.ac.uk) listed over 2800 individual reports of *F8* variants, including all insertion/deletions and single-base DNA replacements, whether directly submitted to the database or derived from journal reports. The overall incidence of hemophilia A does not vary appreciably between different ethnic groups. All classes of defects can result in severe disease. However, the single most clinically important defect is a gene rearrangement (an inversion) involving *F8* intron 22 (see below), which results in approximately 50% of all severe disease cases worldwide. Inversions are omitted from the database as they are almost entirely identical both genetically and by phenotype, and therefore highly redundant. Apart from the intron 22 inversion there are few repetitive mutations, with most genetic defects caused by private mutations. This indicates a high degree of spontaneous mutations in the *F8* gene. Studies in small populations have occasionally shown a preponderance of a single mutation, suggesting a founder effect. But these are often found with mild disease and it is then unclear whether they are causative or act as a marker for a disease-associated haplotype. For full details of the other categories readers should consult the database. A summary of mutations listed in the online database is given in Table 3.1.

Gene rearrangements

As noted above, gross gene rearrangements of *F8* consist almost entirely of a unique inversion whose mechanism was described in 1993 [11,12] and which is now known to be responsible for approximately 50% of all cases of severe hemophilia A. Prior to these studies, PCR amplification of all 26 *F8* exons detected mutations in only about 50% of cases of severe hemophilia. However, reverse transcription (RT)-PCR of *F8* mRNA from most of the remaining severe cases showed that no amplification was possible between exons 22 and 23, suggesting a rearrangement within the *F8* gene in this region.

Unusually, the intron separating exons 22 and 23 (IVS22) contains a CpG island associated with two additional transcripts, originally termed *F8A* [13] and *F8B* [14]. *F8B* is a

Table 3.1 Summary of mutation data available from analysis of 2853 individual reports in the online hemophilia A database at http://europium.csc.mrc.ac.uk

		Clinical severity where reported				Inhibitor status where reported	
Hemophilia A mutations		Severe (%)	Moderate (%)	Mild (%)	Variable (%)	Positive (%)	Negative (%)
Total unique mutations	1221	718 (67)	123 (11)	212 (20)	23 (2)	171 (19)	727 (81)
Unique single-base variants	809	369 (52)	105 (15)	206 (29)	23 (3)	78 (13)	513 (87)
Missense	583	204 (40)	92 (18)	187 (37)	23 (5)	44 (10)	391 (90)
Stop	131	112 (97)	4 (3)	0 (0)	0 (0)	31 (31)	69 (69)
Splice	95	53 (66)	9 (11)	19 (23)	0 (0)	3 (5)	53 (95)
Unique insertions	80	67 (91)	5 (7)	2 (3)	0 (0)	16 (26)	46 (74)
Small (<50 bp)	75	63 (92)	5 (7)	1 (1)	0 (0)	15 (25)	45 (75)
Large (>50 bp)	5	4 (80)	0 (0)	1 (20)	0 (0)	1 (50)	1 (50)
Unique deletions	332	282 (95)	13 (4)	4 (1)	0 (0)	77 (31)	168 (69)
Small (<50bp)	197	157 (93)	9 (5)	4 (2)	0 (0)	31 (22)	113 (78)
Large (>50bp)	135	125 (97)	4 (3)	0 (0)	0 (0)	46 (46)	55 (55)

The distribution of these mutations among severe, moderate, and mild disease, and relation to inhibitor status where known, are given. *F8* inversions invariably result in severe disease, but are not included in the database because of their high redundancy: inversions are responsible for about 45% of all severe disease.

transcript of 2.5 kb and is transcribed in the same direction as the *F8* gene, using a private exon plus *F8* exons 23–26. *F8A* is transcribed in the opposite direction to the *F8* gene; furthermore, two additional copies of *F8A* were found approximately 300 and 400 kb telomeric to the *F8* gene [13]. Thus, the large majority of the "missing" cases of severe hemophilia were explained by homologous recombination between the 9.5-kb intronic sequence (now termed *int22h-1*) and one of the two extragenic homologs of this sequence (*int22h-2* and *int22h-3*). The recombination occurs during the meiotic division of spermatogenesis, resulting in a large inversion and translocation of the gene sequence including exons 1–22 away from exons 23–26 (Figure 3.2a and b). Of these two common types of intron 22 inversion, the distal homolog is responsible for the majority of the severe hemophilia A inversion cases, while crossover with the proximal copy results in a further minority of cases [15].

Most laboratories now carry out initial screening for the intron 22 inversion in all cases of severe hemophilia A. Whilst Southern blotting provides a relatively laborious screening method, it is able to detect some rarer, nonstandard gene rearrangements involving intervening sequence (IVS) 22. In most centers detection is now performed more rapidly by a long-range PCR method [16].

Even with the recognition of the IVS22 rearrangements a residual 5% (approximately) of severely affected hemophiliacs with no known defect remained. Most of these cases were found to be caused by a different inversion resulting from recombination between a 1.0 kb sequence in intron 1 (*int1h-1*) and a homologous sequence *int1h-2* approximately 140 kb upstream of *F8* [17]. Recombination of these sequences results in separation of the *F8* promoter–exon 1 sequence from the remainder of the *F8* gene (Figure 3.2a and c): this inversion may account for approximately 2–5% of severe hemophilia A cases.

Anti-FVIII inhibitor development is a significant complication in hemophilia A: 21% of intron 22 inversion cases have demonstrated inhibitors [18], a rate higher than the average across all mutations but lower than that in cases caused by large deletions or nonsense mutations (see below).

Single-base substitutions in the *F8* gene

Substitution of single bases in *F8* exons may result in amino acid substitutions ("missense" variants) or the introduction of stop codons causing premature peptide chain truncation ("nonsense" variants). In addition, single-base substitution at mRNA splicing sites (at the intron–exon boundaries) can result in splice variants, which may or may not also include an amino acid alteration.

As of 2007, there were over 1900 individual reports of single-base substitutions in the hemophilia A database, with over 800 *unique* variants listed: the actual reports are far too numerous to show here (even condensed to one line per unique mutation), and readers may access the database directly for phenotypic information. This includes FVIII clotting activity and circulating antigen levels, clinical severity, anti-FVIII inhibitor status, and journal reference (where available) for each report. Some overall analysis, however, is presented here (see Table 3.1).

The unique single-base variants are made up of 583 unique missense mutations (i.e., resulting in an amino acid substitution), 131 unique stop mutations, and 95 unique splice variants. The distribution by exon is given in Table 3.2. Exon 14

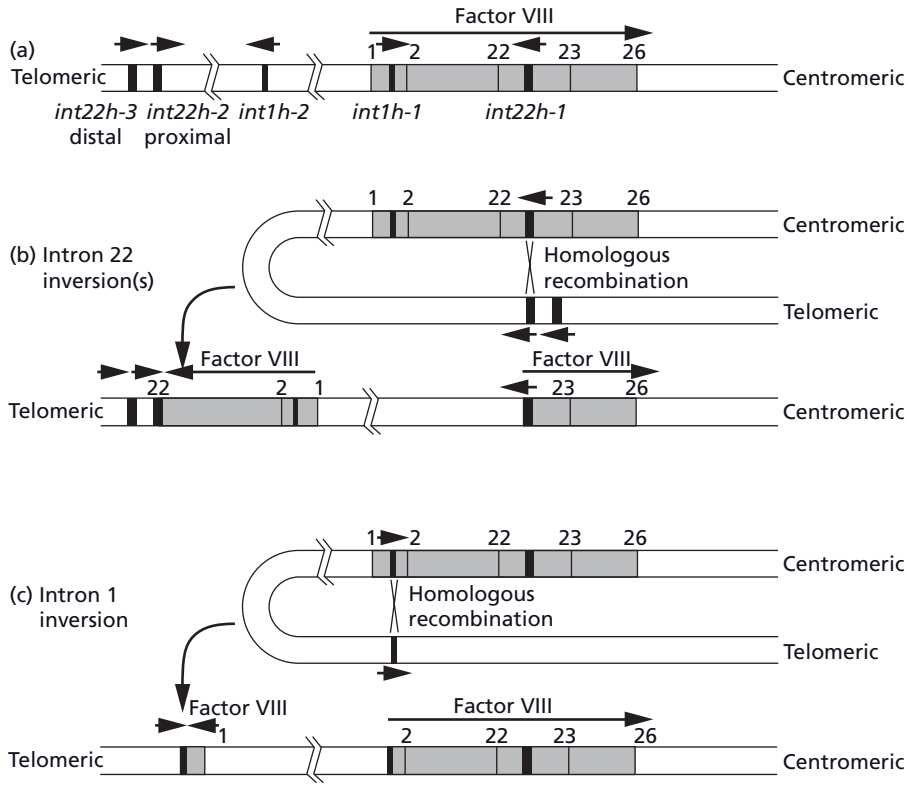

Figure 3.2 Simplified representation of the gene inversion mechanisms resulting in severe hemophilia A, involving sequences in introns 1 and 22 of the *F8* gene. Recombination between homologous sequences in intron 22 and ~400 kb telomeric to the gene leads to separation of exons 1–22 from exons 23–26, with the former sequence inverted and relocated to the site of the telomeric homologous sequence (a and b); alternatively, recombination between the intron 1 sequence and its telomeric homolog results in relocation and inversion of exon 1 (a and c).

contains approximately 10 times as much coding sequence as the other exons and has a much higher burden of causative mutations generally. Missense mutations are actually very poorly represented per kilobase of coding sequence, reflecting the dispensability of the B domain for functional activity.

Table 3.1 gives some stratification of unique single-base mutations by clinical severity and anti-FVIII inhibitor status. While missense mutations and splice variants are associated with all disease severities, stop mutations (as expected) result almost exclusively in severe disease (97% of cases).

In 591 single-base cases in which inhibitor status is known, 78 are reported as inhibitor positive (13%, broadly in accord with published studies on inhibitor incidence) with about 70% of these associated with severe disease. Stop mutations result in a much higher proportion of cases with positive inhibitor status (31 of 100) in comparison with the missense mutation group, in which only 10% (44 of 435) are inhibitor positive, or the splice variant group, in which only 3 of 56 cases had inhibitor development. This extremely low incidence is unexplained. Remarkably, of the 31 unique stop mutations associated with the generation of anti-FVIII antibodies *in vivo*, only four are found in the first 13 exons, and none at all in exons 1–7. The reasons for this highly skewed distribution of inhibitor-positive cases relative to the position of the stop mutation in the FVIII mRNA are unknown, although various hypotheses have been formulated (see Chapters 8–10).

Although the large majority of unique single-base mutations have been reported only once, with over 1900 individual missense reports in the database, it is obvious that many other single-base mutations occur in multiple reports (24 unique mutations have been reported independently on 10 occasions or more, with six of those reported independently over 25 times). This suggests some enhanced predisposition to replication errors at the local chromatin level, whether caused by the well-known CpG dinucleotide effect, by specific sequence motifs (e.g., "runs" or repetitions of a particular base), or by other local factors.

Frequently, both the clinical phenotype and FVIII activity measurement are highly variable within the cases for a single mutation. For example, the mutation Arg2016Trp has been reported 26 times, with clinical severity varying from severe to mild, and FVIII activity from <1% to 5% of normal plasma values. The variability in these multiple reports suggests that additional factors besides the defined mutation in the *F8* gene influence FVIII levels. It is now well known that mutations in at least two genes coding for proteins involved in glycoprotein trafficking in the endoplasmic reticulum (ER) and Golgi apparatus can result in reductions in the secreted levels of both FVIII and FV, resulting in the rare disorder of combined FVIII and FV deficiency [19]. FVIII deficiency may also be seen in von Willebrand disease, particularly the 2N subtype. However, it is likely that there are additional unknown factors, both genetic and environmental.

Now that accurate structural information about normal FVIII and its domains is available it is possible to make interpretations as to the cause of hemophilia at the level of

Table 3.2 Summary of the distribution of mutations in the online database by *F8* exon.

F8 exon		Point mutations			Deletions		Insertions
Number	Size (bp)	Missense	Nonsense (stop)	Splicing	Small	Large	
1	343	15	2	4	2	na	1
2	122	10	1	5	8	na	4
3	123	25	0	6	5	na	0
4	213	35	7	4	1	na	1
5	69	13	1	10	4	na	1
6	117	11	2	6	4	na	2
7	222	38	5	4	8	na	1
8	262	27	6	1	8	na	1
9	172	27	3	5	7	na	3
10	94	10	3	3	5	na	0
11	215	36	1	4	2	na	1
12	151	20	5	4	2	na	1
13	210	32	3	2	7	na	3
14	3106	43	52	6	77	na	39
15	154	19	2	3	4	na	0
16	213	26	7	2	7	na	0
17	229	28	3	2	6	na	5
18	183	34	5	1	5	na	4
19	117	16	1	8	4	na	4
20	72	8	1	0	2	na	2
21	86	9	5	1	0	na	1
22	156	19	5	4	3	na	1
23	145	30	1	4	7	na	0
24	149	13	5	4	3	na	2
25	177	13	2	2	7	na	3
26	1959	26	3	0	9	na	0
Total		583	131	95	197	135	80

Total unique single base (point) mutations: 824. Total unique mutations of all types: 1236.
na, not applicable.

protein structure. The large group of missense mutations in the database might be expected to provide a fertile area for analysis, correlating FVIII function with protein structure, and increasing our understanding of "molecular pathology" in hemophilic mutation cases. However, in order to make interpretations about either FVIII function in plasma, or the ability of a variant molecule to be secreted, it is necessary to know circulating FVIII antigen levels as well as activity levels.

Thus, a hemophilic missense mutation associated with a plasma activity of, for example, 5% of normal may result either from a functionally normal molecule that is poorly secreted or unstable and thus circulates at the level of 5% (often termed "CRM-negative") or from a dysfunctional molecule present at a normal 100% antigen level ("CRM-positive"). In the absence of a FVIII antigen measurement, the differentiation of these two possibilities at the level of protein structure will be very difficult, since in the former case one seeks to explain the effect of the amino acid substitution on secretion or stability in plasma, while in the latter the substitution will affect the procoagulant function of the variant protein.

Unfortunately, data on plasma FVIII antigen level (in addition to FVIII activity) are available for only a minority of the unique missense reports despite the availability of commercial enzyme-linked immunosorbent assay (ELISA) kits. In addition, for two-thirds of cases where antigen is reported, the levels are broadly in agreement with activity, indicating that the hemophilic mutations result in defective secretion or instability of an otherwise functionally active cofactor. Since we understand the mechanisms of protein folding, chaperoning, secretion, and clearance so poorly, it is extremely difficult to make mechanistic explanations of why these amino acid changes result in low plasma levels in this group. Possibilities include mutations abolishing existing cysteine residues known to be involved in disulfide bridge formation, introducing new cysteine residues which may promote novel illegitimate disulfide bond formation, or mutations at, or near, site(s) of

binding to VWF, which may result in defective association with the plasma carrier for FVIII.

In the remaining one-third of cases in which plasma activity and antigen levels are both known, FVIII antigen levels are essentially normal (or only mildly reduced) while activity levels are grossly reduced or even undetectable. This indicates that the mutation causes hemophilia by generation of a functionally inactive molecule that circulates normally. This smaller class of missense mutations gives strong clues as to which amino acids are crucial for functional interactions in this enormous molecule, and attempts have been made to interpret CRM-positive mutations in terms of structure–function relationships [20].

Rationalization of the effects of some of these missense mutations is fairly simple: for example, modifications of proteolytic sites known to be required for FVIII activation (Arg391Cys/His, Ser392Pro/Leu, Arg1708Cys) all result in normal circulating FVIII protein levels with functional activity in the range 12% or below. Other mutations would be expected to have their effect by modifying interaction with one of the ligands of FVIII (e.g., VWF, factor IXa, phospholipid membrane), resulting in reduced functional activity. For example, three mutations with very low FVIII activity and normal antigen levels are associated with N-glycosylation variants. Ile585Thr leads to a new glycosylation (Asn583) at a FIXa binding site; Met1791Thr predicts a new N-glycosylation at Asn1789, also close to a FIXa-binding region; and, unexpectedly, loss of an N-glycosylation site at Asn601 resulting from the Ser603Arg mutation also results in a dysfunctional protein.

Mutations impacting the stability of the heterotrimeric form of activated FVIII (FVIIIa) may give rise to hemophilic consequences. A number of mutations that are widely separated in the linear sequence of FVIII cluster at the interdomain interfaces and produce discrepancies in measured FVIII activity depending on which laboratory assay is used. FVIII activity is normally measured by either a one-stage or two-stage clotting assay or a chromogenic assay. These are discussed in more detail in Chapter 38 but in many laboratories the chromogenic and two-stage assays are considered to measure FVIII activity by a similar methodology. In the vast majority of hemophiliacs the FVIII activity is the same whichever assay is used, but in a small, but distinct, group of cases of mild or moderate hemophilia caused by missense mutations the results vary. This can cause problems in clinical management with uncertainty as to which assay truly reflects the patient's phenotype. In classical "assay discrepancy" the one-stage assay produces higher activity levels than the two-stage. There are now some 15 unique mutations in this group with the one-stage assay producing levels that are up to fivefold higher. Among the first of this class of mutants that were described are Ala303Glu, Ser308Leu, and Arg550Cys. In a rarer group with "inverse assay discrepancy" the results with the one-stage assay are lower. There are at least five unique mutations in this group (personal communication from Dr. Mike Makris, Sheffield Haemophilia and Thrombosis Centre). In both situations the chromogenic tends to agree with the two-stage value.

Some explanation for the mechanism behind this phenomenon can be derived from mapping of the mutations in the crystal structure of FVIII (Figure 3.3). Although the structure is of nonactivated FVIII rather than FVIIIa, it does provide an indication of the position, and interaction between, adjacent domains. The majority of the mutations occur at the interface between the A2 domain and the A1 or A3 domains. As the A2 domain is a separate polypeptide chain that is held within the complex by electrostatic forces, mutations in these residues are likely to affect the strength of that interaction. The chromogenic and two-stage assays include a longer incubation time than the one-stage assay, leading to greater dissociation of the A2 domain in mutants with weakened attachment. Prolonging the incubation time in the one-stage assay leads to lower levels, confirming that this is the likely mechanism. However, some of the mutants are at the interface between the A3 and C1 domains which are covalently attached, indicating that destabilization of interactions between other domains can also affect function. It is generally felt that the two-stage or chromogenic values correlate better with the clinical phenotype and it is noteworthy that in the few cases where thrombin generation has been measured the results correlate better with these assays.

The mechanism of inverse assay discrepancy is likely to be more diverse. The first mutant in this group that was fully studied (Tyr365Cys) is close to a thrombin cleavage site, with a resulting reduction in the rate of activation by thrombin. In this case the assays with longer incubation times allow for more mutant protein to become activated, producing higher levels.

Although these mutants represent a small group, it is becoming clear that reliance on the one-stage FVIII assay as the sole functional test in the diagnosis of nonsevere hemophilia A might lead to the diagnosis being missed.

It is a reasonable assumption that approximately two-thirds of all hemophilic missense mutations will cause the phenotype by defective FVIII secretion or stability while the remaining one-third generate a dysfunctional molecule. However, only performance of FVIII antigen assays allows assignment of a particular mutation into the appropriate category of molecular pathology: in the absence of these data, apparently sophisticated analyses of interspecies residue conservation or structural modeling may be misleading.

Sequence insertions and deletions

The hemophilia A mutation database lists both *F8* insertions and the more numerous deletions. *F8* deletions are divided for convenience into large (>50 bp) and small (<50 bp).

Sequence insertions

There are 158 individual reports of insertions associated with hemophilia A; however, these are composed of just 80 unique

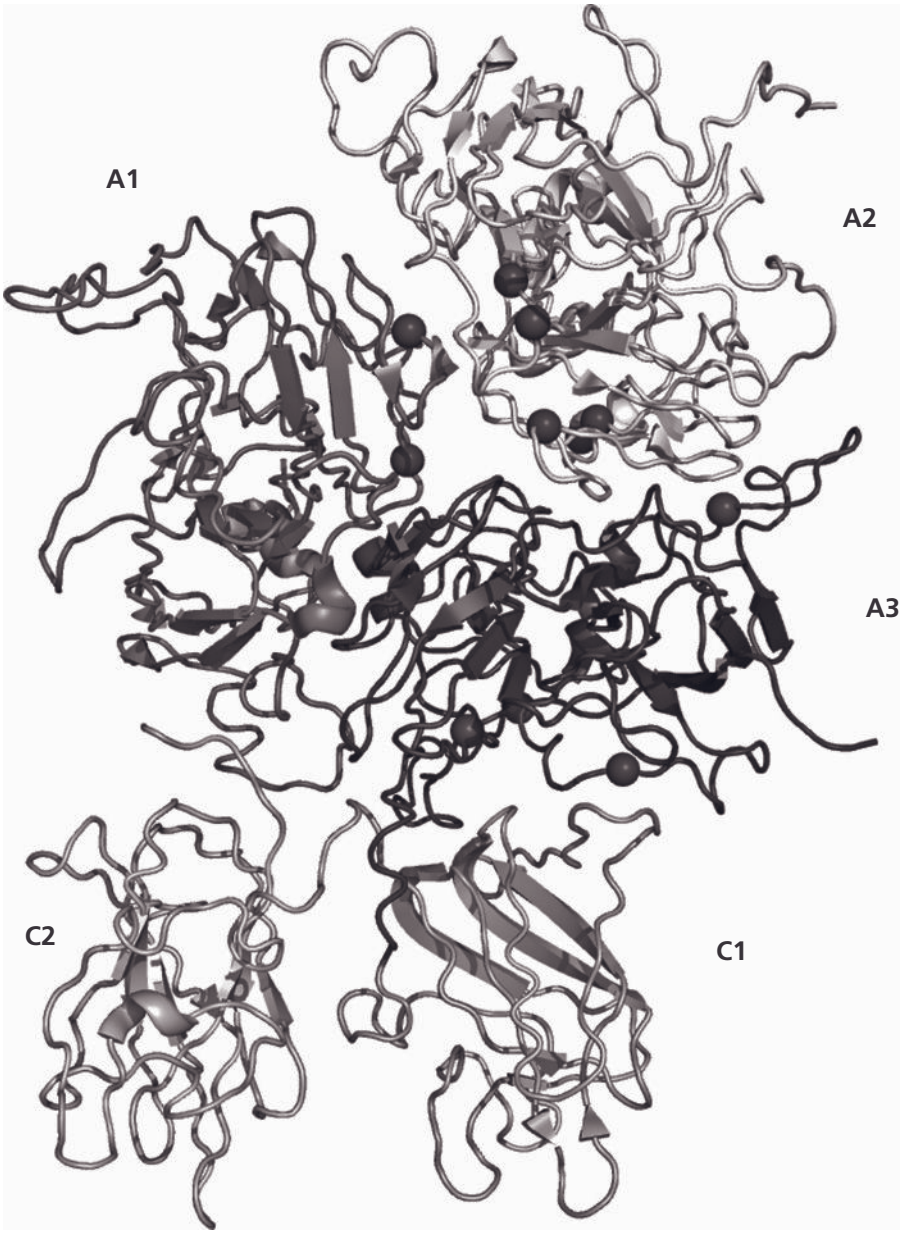

Figure 3.3 Ribbons representation of the crystal structure of B-domain-deleted factor VIII. The positions of mutations associated with classical assay discrepancy are shown by red spheres. These cluster in two groups: at the interface between the A2 domain and the A1 and A3 domains and at the interface between the A3 and C1 domains. PDB ID: 2R7E. (See also Plate 3.3.)

insertion events, the vast majority of which are very short (less than 10 bp), with a very small number of larger insertions such as long interspersed elements [21] or Alu repeats [22]. Most of the repetitious reports consist of insertions of an additional adenine base at the site of a run of adenines; for example, there are more than 20 separate unrelated cases of insertion of an A into a run of eight As at codons 1458–1460, and a further 12 cases of insertion of A into a run of nine As at codons 1210–1213. Runs of As in the *F8* cDNA are relatively common, and these insertions (and small deletions, see below) result from DNA polymerase slippage during replication.

Although the vast majority of insertions cause frame-shifts resulting in severe hemophilia (Table 3.1), a small number of "A-run" insertion cases are associated with low but measurable FVIII activity levels and only moderate (or even mild) clinical severity. This probably results from a small percentage of normal mRNA molecules being produced by "corrective" slippage errors on the mutant template during transcription.

Of the 80 unique insertions, 62 have inhibitor status reported, with 26% of cases inhibitor positive (Table 3.1)—a very similar percentage to that found in single-base stop mutations (see above), as may be expected.

Sequence deletions

Small deletions subgroup (<50 bp)

There are 340 individual small deletion reports in the database, composed of 197 unique small deletions, of which 55%

(108/197) are of single bases. As with small insertions (see above) almost all the multiple reports are of deletions of a single A in a run of As: For example, there are 35 separate reports of an A deletion in a run of nine As at codons 1210–1213, which is also a hotspot for single-base insertions (see above).

Small deletions generally cause frame-shifts and are almost all associated with severe disease; however, as with small insertions, there are a small number of moderate or mild cases, often associated with "A-runs" but also with in-frame deletions. Overall inhibitor development is lower than expected, at 22% of all unique cases with known status, compared with values of around 30% for insertion frame-shifts and nonsense point mutations.

Large deletions subgroup (>50 bp)

There are 135 individual reports in the database, probably comprising close to that number of unique large deletions (detection methods are fairly imprecise so it is difficult to compare different reports where the deleted sequence may be simply given as "10 kb" or "exons 1–5"): the deletions range from just a few hundreds of bases up to more than 210 kb, deleting the entire gene, and are responsible for about 5% of severe hemophilia A cases.

Large deletions in the *F8* gene almost invariably give rise to clinically severe disease with no measurable FVIII activity or antigen. Unexpectedly, however, there are four independent reports of clinically moderate disease (two quoting low but measurable FVIII activity levels, one also a normal FVIII antigen level), all associated with exon-skipping deletions toward the C-terminus of the FVIII protein, involving exon 22, exons 23–24, and exon 25. Remarkably, significant or even normal secretion of hypoactive FVIII lacking C-terminal amino acid sequences may occur in these cases, alleviating the clinical severity.

There is a very high level of inhibitor development (46%) in this subgroup of cases (46/101)—far higher than that found for the small deletion subgroup (22%): clearly, there is a relationship between the size of deletion and the likelihood of inhibitor development. Grouping all deletions yields an overall inhibitor development rate of 31% (77 of 245; Table 3.1), similar to the nonsense mutation group (31%).

Conclusion

After about 25 years of mutation hunting in hemophilia A, the technology of detection has reached the point where, in large centers with substantial cohorts of patients, causative mutations can be found in around 95% of cases. The remaining small percentage of undetermined genetic causes may suggest that there are unknown gene rearrangements still to be found, or that there are other causes outside the *F8* gene; however, there remains an understandable impetus for afflicted families to have their mutation defined for prenatal diagnosis.

Rather surprising, considering the number of years over which mutations have been accumulating in the database, is the fact that novel mutations continue to be added at an undiminished pace, and there are several large centers which have not as yet submitted their own mutation lists. The number of missense mutations causing clinical disease as a fraction of the length of the FVIII primary sequence currently stands at just over 32% (approximately 720 of 2232). How many more disease-causing mutations exist within this remarkable protein is yet to be determined.

Public databases

The Haemophilia A Mutation, Structure, Test and Resource Site (HAMSTeRS): http://europium.csc.mrc.ac.uk/

The Human Gene Mutation Database: http://www.hgmd.cf.ac.uk/ac/index.php

Mutation nomenclature

Numbering in this chapter follows the guidelines of the Human Genome Variation (HGV) Society. +1 refers to the "A" of the ATG translation start codon for nucleotide numbering and the methionine residue that this codes for in peptide numbering. Most earlier publications use a peptide numbering based on the mature protein: subtraction of 19 from the amino acid numbering given here generates the previous commonly used numbering.

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References

1 Rotblat F, Goodall AH, O'Brien DP, Rawlings E, Middleton S, Tuddenham EG. Monoclonal antibodies to human procoagulant factor VIII. *J Lab Clin Med* 1983; **101**: 736–46.
2 Gitschier J, Wood WI, Goralka TM, *et al.* Characterization of the human factor VIII gene. *Nature* 1984; **312**: 326–30.
3 Ngo JC, Huang M, Roth DA, Furie BC, Furie B. Crystal structure of human factor VIII: Implications for the formation of the factor IXa–factor VIIIa complex. *Structure* 2008; **16**: 597–606.
4 Shen BW, Spiegel PC, Chang CH, *et al.* The tertiary structure and domain organization of coagulation factor VIII. *Blood* 2008; **111**: 1240–7.
5 Toole JJ, Knopf JL, Wozney JM, *et al.* Molecular cloning of a cDNA encoding human antihaemophilic factor. *Nature* 1984; **312**: 342–7.

6 Vehar GA, Keyt B, Eaton D, *et al.* Structure of human factor VIII. *Nature* 1984; **312**: 337–42.

7 Kane WH, Davie EW. Cloning of a cDNA coding for human factor V, a blood coagulation factor homologous to factor VIII and ceruloplasmin. *Proc Natl Acad Sci USA* 1986; **83**: 6800–4.

8 Stubbs JD, Lekutis C, Singer KL, Bui A, Yuzuki D, Srinivasan U, Parry G. cDNA cloning of a mouse mammary epithelial cell surface protein reveals the existence of epidermal growth factor-like domains linked to factor VIII-like sequences. *Proc Natl Acad Sci USA* 1990; **87**: 8417–21.

9 Johnson JD, Edman JC, Rutter WJ. A receptor tyrosine kinase found in breast carcinoma cells has an extracellular discoidin I-like domain. *Proc Natl Acad Sci USA* 1993; **90**: 5677–81.

10 Eaton D, Rodriguez H, Vehar GA. Proteolytic processing of human factor VIII. Correlation of specific cleavages by thrombin, factor Xa, and activated protein C with activation and inactivation of factor VIII coagulant activity. *Biochemistry* 1986; **25**: 505–12.

11 Naylor J, Brinke A, Hassock S, Green PM, Giannelli F. Characteristic mRNA abnormality found in half the patients with severe haemophilia A is due to large DNA inversions. *Hum Mol Genet* 1993; **2**: 1773–8.

12 Lakich D, Kazazian HH, Jr., Antonarakis SE, Gitschier J. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. *Nat Genet* 1993; **5**: 236–41.

13 Levinson B, Kenwrick S, Lakich D, Hammonds G, Jr., Gitschier J. A transcribed gene in an intron of the human factor VIII gene. *Genomics* 1990; **7**: 1–11.

14 Levinson B, Kenwrick S, Gamel P, Fisher K, Gitschier J. Evidence for a third transcript from the human factor VIII gene. *Genomics* 1992; **14**: 585–9.

15 Antonarakis SE, Rossiter JP, Young M, *et al.* Factor VIII gene inversions in severe hemophilia A: Results of an international consortium study. *Blood* 1995; **86**: 2206–12.

16 Liu Q, Nozari G, Sommer SS. Single-tube polymerase chain reaction for rapid diagnosis of the inversion hotspot of mutation in hemophilia A. *Blood* 1998; **92**: 1458–9.

17 Bagnall RD, Waseem N, Green PM, Giannelli F. Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe hemophilia A. *Blood* 2002; **99**: 168–74.

18 Oldenburg J, El-Maarri O, Schwaab R. Inhibitor development in correlation to factor VIII genotypes. *Haemophilia* 2002; **8**(Suppl. 2): 23–29.

19 Zhang B, McGee B, Yamaoka JS, *et al.* Combined deficiency of factor V and factor VIII is due to mutations in either LMAN1 or MCFD2. *Blood* 2006; **107**: 1903–7.

20 McGinniss MJ, Kazazian HH, Jr., Hoyer LW, Bi L, Inaba H, Antonarakis SE. Spectrum of mutations in CRM-positive and CRM-reduced hemophilia A. *Genomics* 1993; **15**: 392–8.

21 Kazazian HH, Jr., Wong C, Youssoufian H, Scott AF, Phillips DG, Antonarakis SE. Haemophilia A resulting from de novo insertion of L1 sequences represents a novel mechanism for mutation in man. *Nature* 1988; **332**: 164–6.

22 Sukarova E, Dimovski AJ, Tchacarova P, Petkov GH, Efremov GD. An Alu insert as the cause of a severe form of hemophilia A. *Acta Haematol* 2001; **106**: 126–9.

4 Phenotypic–genotypic relationship

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Introduction

Phenotypic differences between patients with severe hemophilia have gained a large interest. Elucidating the origin for differences in phenotype in a disease caused by a single gene mutation has the possibility to shed more light on factors that influence the hemostatic balance and also to concomitantly discover new treatment modalities. In 1984 the molecular basis of hemophilia A was unraveled [1]. This led to a large interest to establish the mutation that causes hemophilia. It was first hypothesized that the differences in bleeding pattern were explained by mutation type only. However, even in patients with inversions, phenotypic differences were observed. It is increasingly obvious that even in a monogenic disease the phenotype is determined by more than a single factor. This phenomenon has also been demonstrated in other hematologic diseases such as sickle cell anemia [2].

Further studies on the phenotype in severe hemophilia are interesting because the clinical symptoms are well established and only the severity of the symptoms varies between patients. Moreover, the crucial role of factor VIII/IX in the coagulation cascade gives possibilities to unravel other factors important for hemostasis.

The aim of this chapter is to consider different hypotheses that may lead to differences in hemostasis and as a consequence to the heterogeneity of phenotype.

Bleeding patterns and severity of hemophilia

In hemophilia the clinical diagnosis has been largely determined by the differences in bleeding type associated with the level of coagulation factor VIII or IX activity. The phenotypic differences are caused by a slight elevation of the factor VIII/IX baseline from <0.01 IU/mL for severe hemophilia, up to >0.01 and ≤0.05 IU/mL for moderate hemophilia, are large [3]. In cohorts with hemophilia followed before the era of modern hemophilia treatment, the phenotypic range covers the whole spectrum from very frequent joint and muscle bleedings that lead to crippling arthropathy and death at a mean age of 20 years to limited clinical problems in patients with moderate hemophilia [3].

Patients with severe hemophilia will start bleeding earlier; typically at the age of 6–8 months when the child becomes more active [4]. Within patients with severe hemophilia, the age at onset of joint bleeding is variable and has been recorded to be between 6 months and almost 6 years [4,5] (Figure 4.1). Patients with moderate hemophilia express the most varied bleeding pattern, which can vary between almost no bleeding up to severe muscle and joint bleedings, which will prompt the use of prophylaxis, as in patients with severe hemophilia [6–8]. The estimation of the baseline level is crucial and should be performed at least three times in a well-established laboratory. Within patients with moderate hemophilia, bleeding pattern appears related to baseline factor VIII levels [7]: patients with >2–3% factor VIII rarely bleed.

The observation, made more than 40 years ago, that levels of factor VIII/IX of only 2–3% were associated with fewer bleeds was the reason in Sweden to start prophylaxis and change the phenotype of a patient with severe hemophilia to a moderate one [9].

Bleeding pattern in severe hemophilia

Because of the consequences for dosing prophylaxis, together with the high cost of prophylactic treatment, investigators took a large interest in identifying other factors in patients with severe hemophilia that could potentially influence phenotype.

An available parameter that is mostly independent of treatment is the age at onset of bleeding, especially the age at the first joint bleed. In addition, several prospective studies have documented that 10% of patients with severe hemophilia do not suffer frequent joint bleeds and have no or minimal arthropathy even with limited therapy [10–12]. For patients on prophylaxis this parameter is, of course, dependent on treatment, but it can be approximated by studying variation in clotting factor consumption necessary to prevent the patient from bleeding.

However, determining the number of bleeds is difficult, especially in patients on prophylaxis. These patients experience

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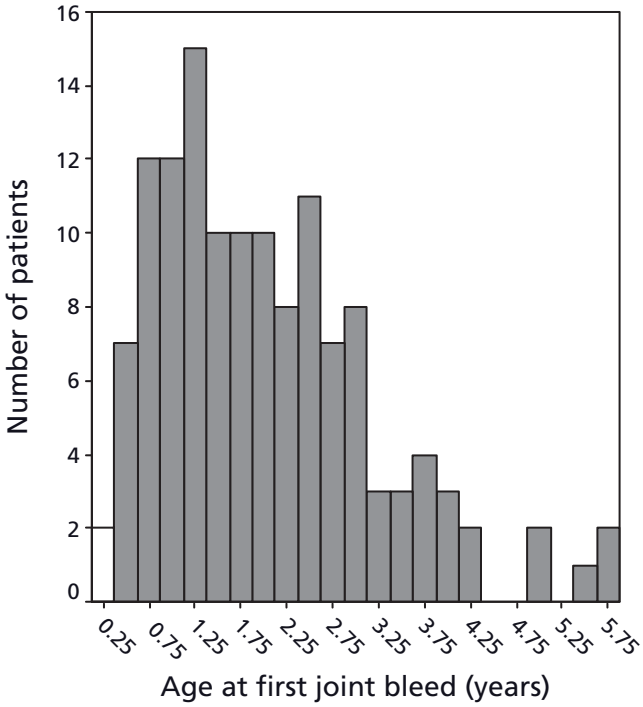

Figure 4.1 Distribution of age at first joint bleed in 132 patients with severe hemophilia. Median 1.8 years, range 0.2–5.8. Reproduced from [5] with permission.

no or limited joint bleeds and will report every event/case of joint pain as a bleeding. In contrast, patients receiving on-demand treatment, and especially those without home treatment, will come into the hospital only with large bleeds and will ignore small joint bleeds. This effect has to be recognized in defining scoring of phenotypes in clinical studies.

For orthopedic outcome, lifelong treatment history and calculations of bleeding and traumatic incidents have to be considered.

In 2001, Fischer *et al.* developed a prognostic score to predict which patients could stop taking prophylaxis in adulthood [13]. This score was based on the age at start of prophylaxis, mean lifetime dose of prophylaxis (IU/kg/week), and mean lifetime joint bleed frequency (number/year). Recently, Schulmann *et al.* presented another scoring system to quantitate the phenotype of hemophilia [14]. This score was based on the annual number of joint bleeds and clotting factor consumption calculated from a 10-year follow-up period, in combination with the World Federation of Hemophilia score for orthopedic outcome. In this study patients with mild, moderate, and severe hemophilia were included. Unfortunately, both scoring systems are highly dependent on treatment regimen (especially dose of prophylaxis) and cannot be used directly in other hemophilia populations.

Several other studies demonstrated that in patients with severe hemophilia a later age at first joint bleeding was associated with a milder (bleeding) phenotype [15,16].

Review of other factors that may influence hemostasis

Several determinants may have an impact on hemostasis and eventually affect the patient's bleeding phenotype. Some are listed below.

Gene defects

Nowadays, it is possible to determine the gene defect in almost all patients with hemophilia [17,18]. A wide range of mutations has been recognized and more will be added in the following years.

More than 50% of patients with severe hemophilia A have inversions in intron 22 [18,19]. As a consequence, they have a complete absence of factor VIII. Theoretically, they form a perfect patient group for studying the causes of variation in phenotype. It is very likely that the substantial variation in bleeding phenotype in this population is caused by factors independent of factor VIII levels. However, even in this monogenetic subgroup a large difference in phenotype has been observed. Although the determination of the gene defect is very important and can classify patients in different diagnostic groups, it has not been proven to be more accurate than the measurement of the factor VIII/IX level.

Hemostatic balance

The blood coagulation cascade is initiated when tissue factor (TF) is exposed to the circulating blood and binds to plasma factor VIIa [20]. The factor VIIa–TF complex triggers a cascade of reactions which ultimately lead to thrombin generation and, consequently, fibrin–platelet clot formation. Factor VIII, together with factor IX, is crucial for the accelerated formation of factor Xa by TF–VIIa complex. Factor VIIIa increases the catalytic activity of factor IXa, which results in approximately 50-fold more efficient conversion of factor X into factor Xa by the intrinsic pathway [20,21].

The coagulation process is downregulated by inhibitors such as antithrombin (AT) and proteins C and S. Defects of these inhibitors and the more common mutation in the factor V gene (*FV Leiden*) can potentially lead to a prothrombotic status. Large cohort studies have demonstrated that thrombosis is a multifactorial event and that only the measurement of thrombotic risk factors simplifies the problem [22].

Moreover, in the last 10 years it has been established that the levels of coagulant and anticoagulant factors in individuals are variable. In addition, levels of coagulation factor will change during the lifetime of individual patients. This confirms the complexity of the relationship between single genetic mutation and biology [23]. Several studies have searched for the influence of prothrombotic mutations in patients with hemophilia. Already, in 1996, the association between *FV Leiden* and a milder phenotype was observed [24]. In a large pediatric study the prevalence of prothrombotic risk factors

in children with severe hemophilia A did not differ from previously reported data in the normal population [16]. However, the first symptomatic bleeding leading to diagnosis in children with severe hemophilia occurred at a median age of 1.6 years (range 0.5–7.1 years) in children having prothrombotic risk factors. This was significantly later than in hemophilic patients without risk factors [0.9 years (range 0.1–4.0; $P = 0.01$)] [16].

In an attempt to find the determinants of clinical phenotype in the clotting cascade, Van Dijk *et al*. studied 42 patients with severe hemophilia [25]. These patients were selected from the extreme ends of a well-defined single-center cohort of 214 patients with severe hemophilia, with lifelong data on treatment and outcome available (Table 4.1). Standardized laboratory tests were performed after a wash-out period of a minimum of 72 h. Table 4.1 shows the large differences in phenotype between both groups. The age at first joint bleed varied between a median age of 1 year for the severe group versus 4.6 years for the mild group. Moreover, significant differences ($P < 0.01$) for the total number of joint bleeds and annual clotting factor consumption were observed.

Table 4.1 Patients ($n = 42$) with a severe versus a mild phenotype, selected from the extreme ends of 285 patients with severe hemophilia.

Phenotype	Severe		Mild	
Number of patients	21		21	
Intron 22 inversion, *n* (%)	12 (57)		11 (52)	
	Mean	*Interquartile range*	*Mean*	*Interquartile range*
Age (years)	23	18–42	30	20–40
Age at first joint bleed (years)	1.0	0.4–1.2	4.6	3.6–5.5
Clotting factor use (IU/kg/year)	2214	1758–2695	1154	628–1993
Joint bleeds (number per year)	3.5	1.6–4.4	1.8	1.0–5.5
Joints with Pettersson score <3	0.5	0–2	4.5	4–5
VWF:Ag (%)	122	104–147	114	95–136
VWF:ristocetin (%)	114	91–134	120	95–136
Protein C:act (%)	89	80–100	87	81–99
Protein S:Ag (%)	94	84–109	92	76–106
aPC response ratio	0.97	0.94–1.01	1.00	0.97–1.02

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Interestingly, the proportion of patients with an inversion was similar. A large interindividual variation of coagulation factors was observed. However, no significant differences were found when comparing coagulation factors, prothrombotic factors, or fibrinolytic factors between the two groups.

Thrombin generation

The coagulation system is usually investigated by means of two *in vitro* classic clotting tests, the activated partial thromboplastin time (aPTT) and prothrombin time (PT), which assess only time to initiation of clot formation and do not entirely reflect global hemostatic balance.

Over the last 10 years there has been increasing interest in the application of tests that are able to measure the overall hemostatic potential in a patient. Some studies have suggested that clot waveform analysis may be used to detect differences in phenotype [26]. Studies on the clinical implications of tests for the whole hemostasis have been reviewed recently [27]. Tests such as the thrombin generation test (TGT) measure the course of thrombin formation over time. The most widely used test at the moment is the automated recording of thrombin generation. This test can be performed in both platelet-poor and -rich plasma [28]. Another test to measure the whole hemostatic potential of a patient is the use of the thromboelastogram (TEG) [29]. In this test undiluted plasma, or sometimes whole blood, is used. In a series of 46 patients with hemophilia (34 with hemophilia A and 12 with hemophilia B), a significant correlation between plasmatic factor VIII or IX levels and endogenous thrombin potential (ETP), peak and time to peak obtained by thrombin generation measurement was found [30]. In another study, in which the thromboelastographic principle was used, significant differences in the curves of thrombin generation were observed between patients with the same factor VIII or IX activity level, but correlation of this laboratory variation with the bleeding phenotypes is still lacking [29].

Fibrinolysis

Until now, one study has suggested an association of the fibrinolytic activity with the bleeding phenotype in hemophilic patients. In 21 patients with severe hemophilia the median tissue-type plasminogen activator (TPA) concentration was significantly elevated in patients with a more severe phenotype, as was the activity of the thrombin-activatable fibrinolysis inhibitor (TAFI) [31]. Median activity of the plasminogen activator inhibitor 1 (PAI-1) and the concentration of TPA–PAI-1 complexes were twofold higher than in patients with a less severe phenotype. The hypothesis of different fibrinolytic potential and a changed bleeding phenotype was also tested in a group of patients with severe hemophilia. In this *in vitro* study, the potential of recombinant factor VIIa to downregulate fibrinolysis via activation of TAFI was investigated. It was clear that there was a large intervariability in antifibrinolytic

potential of recombinant factor VIIa between patients [32]. Whether these differences can also explain the heterogeneity of phenotypes has not yet been established.

In parallel with findings in rheumatoid arthritis, it has been suggested that interleukin 10 (IL-10) levels may have a potential protective effect against arthropathy. This may be one of the additional factors explaining the heterogeneity in joint damage [33,34]. It also cannot be ruled out that inflammation influences the levels of these factors, making them likely to be less specific for studying heterogeneity in patients.

Platelets

When platelet function is measured *in vitro*, large interindividual variability is observed [35]. Because platelets have a large impact also on the formation of thrombin it can be speculated that platelets also influence phenotypes in hemophilia. A first study which has undertaken this approach was performed a long time ago [36]. Its results suggested that platelet coagulation activity could have an impact on the bleeding tendency in hemophilia.

Environmental factors

The clinical phenotype also may be influenced by external factors. Risk-taking behavior and physical fitness may play an important role. Although there has been no formal research on the role of physical activity on bleeding pattern, one may hypothesize that patients with a less active lifestyle encounter fewer bleeds. On the other hand, muscle training and participation in sport can sustain joint function and prevent bleeds [37,38].

Discussion

The considerable variability in bleeding patterns within patients with severe hemophilia has prompted studies on factors other than factor VIII that may influence the final outcome of the hemostatic balance in patients with hemophilia.

The most promising determinants of hemostatic balance were the well-known prothrombotic risk factors. A study in mice suggested that factor V Leiden has the ability to improve the hemophilia A or B phenotype, but this effect was mainly evident at the level of the microcirculation following a particular vascular injury [39]. Another interesting study assessed the role of factor V inactivation in relation to activated protein C (aPC) using a mouse model. It was concluded that aPC-resistant factor V had the potential to influence hemophilic bleeding [40].

Although the results of these studies are interesting, they do not provide the full explanation of differences in phenotype. Recently, it was demonstrated after factor VIII substitution that an almost linear relation was observed between the individual factor VIII activities administered to the patients and the activities measured in the plasma samples [41]. However, data obtained with TGT and TEG revealed a high interindividual variation and a very poor correlation to the administered factor VIII activity. It was concluded that to measure the hemostatic effect of factor VIII not only the activity but also the interplay between coagulation factors and cells, especially platelets, should be taken into account.

The role of the fibrinolytic system has been recently largely discovered [42]. This is also an interesting field but has only been scarcely been investigated in hemorrhagic diseases.

Gene defects such as the inversion type and large deletions lead to a total absence of coagulation factor. Since these defects are present in more than 50% of the patients with severe hemophilia, these patients are ideal candidates to study the effects of other parameters. Additional studies on the determinants of phenotype preferably should be undertaken in this genetically homogenous group of patients.

Although it is well known that coagulation assays are difficult to standardize, the benefits of the thrombin generation test in determining a bleeding phenotype still have to be demonstrated. From a clinical point of view, it would be very interesting to predict a patient's bleeding phenotype more accurately. The study of other determinants of coagulation is largely complicated by the large interindividual variance of these factors. Moreover, they not only vary in a patient, but are also influenced by, for instance, inflammation. To study the correlation between different factors large cohort studies are needed, such as performed in cardiology. It can be debated whether this is feasible for a rare disease like hemophilia.

Conclusion

The origin of the large heterogeneity of phenotypes in severe hemophilia is multifactorial. As they produce no factor VIII, patients with severe hemophilia and inversions are ideal candidates to further studies on these parameters. To correlate new factors to phenotypes in patients, large and well-characterized cohorts are needed. Until other parameters have been identified, the heterogeneity of the clinical phenotype may best be predicted by the age at first joint bleed.

References

1 Gitschier J, Wood WI, Goralka TM, *et al.* Characterization of the human factor VIII gene. *Nature* 1984; **312**: 326–30.
2 Beutler E. Discrepancies between genotype and phenotype in haematology: An important frontier. *Blood* 2001; **98**: 2597–602.
3 Bolton-Maggs PH, Pasi KJ. Haemophilias A and B. Review. *Lancet* 2003; **361**: 1801–9.
4 Onwuzurike N, Warrier I, Lusher JM. Types of bleeding seen during the first 30 months of life in children with severe hemophilia A and B. *Haemophilia* 1996; **2**: 137–40.

5 Van Dijk K, van der Bom JG, Fischer K, Grobbee, DE, Van den Berg HM. Variability in clinical phenotype of severe haemophilia; the role of the first joint bleed. *Haemophilia* 2005; **11**: 438–43.

6 Ljung R, Petrini P, Nilsson IM. Diagnostic symptoms of severe and moderate haemophilia A and B. *Acta Paediatr Scand* 1990; **79**: 196–200.

7 Fischer K, Van der Bom JG, Mauser-Bunschoten EP, *et al.* Endogenous clotting factor activity and long-term outcome in patients with moderate haemophilia. *Thromb Haemost* 2000; **84**: 977–80.

8 Uijl IE, Fischer K, Van der Bom JG, Grobbee DE, Rosendaal FR, Plug I. Clinical outcome of moderate haemophilia compared with severe haemophilia. *Throm Haemost* 2008; **99**: 965–8.

9 Nilsson IM, Berntorp E, Lofqvist T, Pettersson H. Twenty-five years experience of prophylactic therapy in severe haemophilia A and B. *J Int Med* 1992; **232**: 25–32.

10 Aledort LM, Haschmeyer RH, Pettersson H. A longitudinal study of orthopaedic outcomes for severe factor-VIII-deficient haemophiliacs. The Orthopaedic Outcome Study Group. *J Intern Med* 1994; **236**: 391–9.

11 Rainsford SG, Hall A. A three-year study of adolescent boys suffering from haemophilia and allied disorders. *Br J Haematol* 1973; **24**: 539–51.

12 Molho P, Rolland N, Lebrun T, *et al.* Epidemiological survey of the orthopaedic status of severe haemophilia A and B patients in France. The French Study Group. *Haemophilia* 2000; **6**: 23–32.

13 Fischer K, Van der Bom JG, Preijs R, *et al.* Discontinuation of prophylactic therapy in severe haemophilia; incidence and effects on outcome. *Haemophilia* 2001; **7**: 544–50.

14 Schulman S, Eelde A, Holstrom G. Validation of a composite score for clinical severity of hemophilia. *J Thromb Haemost* 2008; **6**: 1113–21.

15 Escuriola-Ettingshausen C, Halimeh S, Kurnik K, Schobess R, Wermes C, Junker R, Kreuz W, Pollmann H, Nowak-Gottl U. Symptomatic onset of severe hemophilia A in childhood is dependent on the presence of prothrombotic risk factors. *Thromb Haemost* 2001; **85**: 218–20.

16 Van Dijk K, van der Bom JG, Lenting PJ, *et al.* Factor VIII half-life and clinical phenotype of severe hemophilia A. *Haematologica* 2005; **90**: 494–8.

17 Schwaab R, Oldenburg J, Tuddenham EG, Brackmann HH, Olek K. Mutations in haemophilia A. *Br J Haematol* 1993; **83**: 450–8.

18 Goodeve AC, Peake IR. The molecular basis of hemophilia A: Genotype–phenotype relationships and inhibitor development. *Semin Thromb Hemost* 2003; **29**: 23–30.

19 Oldenburg J. Mutation profiling in haemophilia A. *Thromb Haemost* 2001; **85**: 577–9.

20 Mann KG. Biochemistry and physiology of blood coagulation. *Thromb Haemost* 1999; **82**: 165–74.

21 Mann KG, Butenas S, Brummel K. The dynamics of thrombin formation. *Atheroscler Thromb Vasc Biol* 2003; **23**: 17–25.

22 Bezemer IR, Rosendaal FR. Predictive genetic variants for venous thrombosis: what's new? *Sem Hematol* 2007; **44**: 85–92.

23 Bertina RM. Elevated clotting factor levels and venous thrombosis. *Pathophysiol Haemost Thromb* 2003; **33**: 395–400.

24 Nichols WC, Amano K, Cacheris PM, *et al.* Moderation of hemophilia A phenotype by the factor V R506Q mutation. *Blood* 1996; **88**: 1183–7.

25 Van Dijk K, van der Bom JG, Fischer K, de Groot PG, van den Berg HM. Phenotype of severe hemophilia A and plasma levels of risk factors for thrombosis. *J Thromb Haemost* 2007; **5**: 1062–4.

26 Shima M, Matsumato T, Fukuda K, *et al.* The utility of activated partial thromboplastin time (aPTT) clot waveform analysis in the investigation of hemophilia A patients with very low levels of factor VIII activity (FVIII:C). *Thromb Haemost* 2002; **87**: 436–41.

27 Berntorp E, Salvagno GL. Standardization and clinical utility of thrombin-generation assays. *Sem Hematol* 2008; **34**: 670–82.

28 Dargaud Y, Meunier S, Negrier C. Haemophilia and thrombophilia: an unexpected association! *Haemophilia* 2004; **10**: 319–26.

29 Sorensen B, Ingerslev J. Whole blood clot formation phenotypes in hemophilia A and rare coagulation disorders. Patterns of response to recombinant factor VIIa. *J Thromb Haemost* 2004; **2**: 102–10.

30 Dargaud Y, Beguin S, Lienhart A, *et al.* Evaluation of thrombin generating capacity in plasma from patients with haemophilia A and B. *Thromb Haemost* 2005; **93**: 475–80.

31 Grunewald A, Siegemund A, Grunewald M. Konegan A, Koksch M, Griesshammer M. Paradoxysmal hyperfibrinolysis is associated with a more intensely haemorrhagic phenotype in severe congenital haemophilia. *Haemophilia* 2002; **8**: 768–75.

32 Lisman T, Mosnier LO, Lambert T, *et al.* Inhibition of fibrinolysis by recombinant factor VIIa in plasma form patients with severe hemophilia A. *Blood* 2002; **99**: 175–9.

33 Huizinga TW, Keijsers V, Yanni G, *et al.* Are differences in interleukin 10 production associated with joint damage? *Rheumatology* 2000; **39**: 1180–8.

34 Verhoef CM, Van Roon JA, Vianen ME, *et al.* Interleukin 10 (IL-10), not IL-4 or interferon-gamma production, correlates with progression of joint destruction in rheumatoid arthritis. *J Rheumatol* 2001; **28**: 1960–6.

35 Kunicki TJ, Orchekowski R, Annis P, Honda Y. Variability of integrin alpha 2 beta 1 activity on human platelets. *Blood* 1993; **82**: 2693–703.

36 Walsh PN, Riansford SG, Biggs R. Platelet coagulant activities and clinical severity in haemophilia. *Thromb Diath* 1973; **29**: 722–9.

37 Heijnen L, Mauser-Bunschoten EP, Roosendaal G. Participation in sports by Dutch persons with haemophilia. *Haemophilia* 2000; **6**: 537–46.

38 Harris S, Boggio LN. Exercise may decrease further destruction in the adult haemophilic joint. *Haemophilia* 2006; **12**: 237–40.

39 Butenas S, van't Veer C, Cawthern K, Brummel KE, Mann KG. Models of blood coagulation. *Blood Coagul Fibrinolysis* 2000; **11**(Suppl. 1): S9–13.

40 Schlachterman A, Schuettrumpf J, Liu JH, *et al.* Factor V Leiden improves *in vivo* hemostasis in murine hemophilia models. *J Thromb Haemost* 2006; **4**: 925.

41 Bassus S, Wegert W, Krause M, *et al.* Platelet-dependent coagulation assays for factor VIII efficacy measurements after substitution therapy in patients with haemophilia A. *Platelets* 2006; **17**: 378–84.

42 Rijken DC, Lijnen HR. New insights into molecular mechanism of the fibrinolytic system. *J Thromb Haemost* 2008; **7**: 4–13.

5 Prophylaxis

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Introduction to prophylaxis

The prevention of bleeds by prophylactic clotting factor replacement therapy has now been advocated for almost half a century. It started with the observation that the clinical phenotype of patients with moderate hemophilia was very different from severe hemophilia [1]. Patients with moderate hemophilia and factor VIII/IX activities of 0.01–0.05 IU/mL bled only after trauma and had a fairly normal life expectancy, while patients with severe hemophilia (factor VIII/IX <0.01 IU/mL) had spontaneous severe muscle and joint bleeds and early crippling hemophilic arthropathy, with a life expectancy of only 20 years. Because of this observation, it seemed logical to supply the missing clotting factor in patients with severe hemophilia and increase the level of clotting factor activity above 1%.

Professor Inga Marie Nilsson from Malmö, Sweden, was the first to start prophylactic replacement therapy with cryoprecipitate in boys with severe hemophilia A [2]. The patients who first started on prophylactic therapy were those with frequent bleeds. In these first studies it was demonstrated that the number of bleeds decreased and that these patients lost fewer days from school or work. This Swedish initiative was repeated in the Netherlands by Professor Van Creveld, who also reported a reduction in the number and the severity of bleeds [3]. After the availability of more clotting factors, the group of patients who received prophylaxis was extended. Moreover, it was observed that early prophylaxis was more effective in preventing arthropathy, and that radiologic joint damage could not be reversed by prophylaxis. So, in subsequent years, prophylaxis was started earlier, before the occurrence of joint damage [4,5].

Consequently, "primary prophylaxis," defined as the start of regular continuous treatment before the age of 2 years or after the occurrence of the first joint bleed [6], was recommended. After favorable experience, prophylaxis increasingly became the treatment of choice for boys with severe hemophilia, and was recommended by the American Medical and Scientific Advisory Council (MASAC) [7] and World Health Organization (WHO)/World Federation of Hemophilia (WFH) experts [8].

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However, some stated that evidence from randomized controlled trials (RCTs) was needed to truly establish the benefits of prophylaxis replacement therapy for severe hemophilia. A Cochrane review, focused on results from RCTs only, stated that there was no evidence for the benefits of prophylactic treatment [9] and was avidly debated in the hemophilia community [10]. In 2007, the results of an American multicenter RCT that had started in 1996 were published [11]. In this trial, 65 of 119 screened patients with FVIII <0.03 IU/mL, aged less than 30 months, and with normal joints on physical examination, magnetic resonance imaging (MRI) and X-ray, were randomized to receive prophylaxis (25 IU/kg every other day) or enhanced episodic treatment (40 IU/kg, followed by 20 IU/kg after 24 and 72 h). MRI and radiography were performed at study entry and at age 6 years. Infusion logs, data on emergency room visits, and physical examination scores were collected frequently. Primary endpoints were MRI and X-ray scores at age 6.

In total, 49 patients completed the protocol, and the mean follow-up was 49 months. Patient characteristics and results are shown in Table 5.1.

As expected, outcome was significantly better in the prophylaxis group: patients treated with the enhanced on-demand regimen had a 6.1 times higher relative risk of joint damage on MRI [95% confidence interval (CI) 1.5–24.4] and a 5.2 times higher risk of damage on X-ray (95% CI 0.65–41.5). Differences in number of bleeds experienced and number of factor infusions were even more significant.

As follow-up was only 4 years, the correlation between MRI findings and the total number of bleeds ($r = 0.14$) and physical examination scores ($r = 0.26$) were only weak. The authors concluded that prophylaxis was effective in preventing hemarthroses and structural joint damage detected by MRI in boys with severe hemophilia.

Preliminary results of the 10-year Italian RCT comparing prophylactic (25 IU/kg three times a week) with on-demand treatment in 40 children with severe hemophilia A again showed that prophylaxis reduces the number of joint bleeds (0.2 vs. 1.1 bleeds/month) and arthropathy (71% vs. 26% of patients with zero Pettersson scores at age 12) [12].

Although these RCTs provide unequivocal evidence that prophylactic replacement therapy prevents bleeds, they will never be able to establish the long-term beneficial effects of prophylaxis on arthropathy. It is simply impossible to conduct an RCT in a rare disease with 20 to 30 years of follow-up

Table 5.1 Patient characteristics and results from the American RCT comparing prophylaxis with enhanced on-demand treatment in patients with hemophilia A and factor VIII activity of <0.03 IU/mL.

	Prophylaxis	Enhanced on-demand treatment	*P*-value
Patient characteristics	*N* = 32	*n* = 32	
Mean age (years)	1.6	1.6	0.78
First index joint bleed before enrolment [*n* (%)]	18 (56)	13 (39)	0.17
Outcome	*n* = 27	*n* = 29	
Absence of MRI changes [*n* (%)]	25 (93)	16 (55)	<0.01
Absence of X-ray changes [*n* (%)]	27 (96)	22 (81)	0.10
Joint bleeds (median number/patient/year)	0.20	4.35	<0.01
Total bleeds (median number/patient/year)	1.15	17.13	<0.01
Number of factor infusions (mean)	653	187	<0.01
Occurrence of life-threatening bleeds (number of patients)	0	3	0.24

Data from [11].

Patients received prophylaxis (25 IU/kg every second day) or enhanced episodic treatment (40 IU/kg, followed by 20 IU/kg after 24 and 72 h). Inclusion criteria: factor VIII < 0.03 IU/mL, age < 30 months, maximum two joint bleeds per index joint (ankles, knees, elbows), normal physical examination, normal joint imaging at baseline, and absence of inhibitors.

[13]; this is why observational studies will remain very important in hemophilia.

The optimal treatment regimen for prophylaxis

Even if the benefits of prophylactic replacement therapy are firmly established, the optimum treatment dose remains under discussion. The half-life of clotting factor concentrates is short: for factor VIII it is about 8 h in children and about 12 h in adults, while the mean half-life for factor IX is about 24 h [14]. As a consequence, prophylactic administration is most effective when given frequently. Carlsson *et al*. [15] calculated that daily prophylactic infusions would reduce factor VIII usage by 82%. In addition, efficiency could further be improved by taking the patient's individual pharmacokinetic profile into account. In theory, daily prophylactic infusions would be most cost-effective. In practice, however, the lifelong need for frequent intravenous injections imposes a heavy burden on the patient and his family, and very few patients are willing to take daily prophylaxis.

Prophylactic therapy is mostly given in a dose of 25–40 IU/kg three times per week. This original Swedish protocol aimed at a preinfusion level of >1% to mimic the clinical phenotype of moderate hemophilia (high-dose regimen). In contrast, in the Netherlands, prophylactic dosages are adjusted according to bleeding pattern and aimed at preventing spontaneous joint bleeds. Trough levels are not taken into account. As a result, this strategy is associated with a relatively low clotting factor consumption (intermediate dose regimen). Both Sweden and the Netherlands have followed their cohorts of patients with severe hemophilia longitudinally, routinely collecting data on treatment and outcome. As both cohorts are complete, selection bias plays no role; therefore, the long-term outcome of these cohorts gives information on the long-term effectiveness of these two regimens.

Using routinely collected data, all 128 patients with severe hemophilia without inhibitors, born between 1970 and 1990, treated in Malmö, Sweden, and the Van Creveldkliniek, the Netherlands, were compared [16]. Patients were evaluated at the date of their last routine radiologic examination, and the median follow-up ranged from 9 to 23 years (Table 5.2). In the 1970s, twice-weekly prophylaxis was started earlier in the intermediate-dose group; this was reversed in the 1980s. In the last three treatment years before evaluation, the majority of patients in both cohorts received prophylaxis three times per week, but overall clotting factor consumption was 2.4 times higher in the high-dose regimen. Overall, patients treated with the high-dose regimen had fewer joint bleeds per year. Differences in long-term orthopedic outcome and Pettersson score were small and only significant for the youngest patients. The study showed that both prophylactic regimens are able to prevent arthropathy to a large extent. Further follow-up of both cohorts is currently under way.

When to start prophylaxis

The timing of the initiation of prophylaxis is important: "primary prophylaxis" has been recommended for all boys with severe hemophilia [6].

Recently, it has been suggested that prophylactic treatment should be individualized according to patient characteristics [17,18]. One of the arguments in favor of an individualized approach is the large variability of bleeding patterns in patients with severe hemophilia, as is reflected in the age at first joint bleed. Several studies have reported a wide variation of the age at first joint bleed, ranging from 0.2 to 5.8 years, with medians of 1.6 and 1.7 years [19,20]. It has been suggested that arthropathy is best prevented if prophylaxis is started before the second [21] or third [18] joint bleed, but the benefits of starting before the first joint bleed have not been established. Strong support for an early start of prophylaxis also can be obtained from the Swedish experience [17]. In an analysis of 121 patients with severe hemophilia, it was shown that the age at start of prophylaxis was an independent predictor for the development of arthropathy, but dose and interval of prophylaxis at the start of prophylactic treatment were not.

Table 5.2 Comparison of treatment and outcome according to differently dosed early prophylactic treatment regimens [16].

	Patients born between 1970 and 1979			Patients born between 1980 and 1989		
	Intermediate dose (n = 44)	High dose (n = 24)	P-value	Intermediate dose (n = 42)	High dose (n = 18)	P-value
Age at evaluation (years)	22.7 (20.4–25.3)	17.2 (15.2–20.4)	<0.01	13.5 (10.6–15.7)	9.0 (6.3–13.5)	<0.01
Age at start of prophylaxis two times per week (years)	5.5 (4.2–8.7)	12.1 (9.5–13.6)	<0.01	4.7 (3.7–6.2)	2.1 (1.2–4.6)	<0.01
Treatment and bleeds in the last 3 years						
Frequency of prophylaxis (n/week)	3 (2–3)	3 (2–3)	0.68	3 (2.5–3)	3.3 (3–3.5)	<0.01
Dose of prophylaxis (IU/kg/week)	35 (24–44)	82 (57–90)	<0.01	40 (33–49)	89 (78–107)	<0.01
Annual clotting factor use (IU/kg/year)	1466 (1039–1926)	4301 (3034–4726)	<0.01	2126 (1743–2755)	4616 (4105–5571)	<0.01
Joint bleeds per year (n)	2.5 (1–5.7)	0.5 (0.2–1.8)	<0.01	3.7 (1.7–5)	0.2 (0–0.3)	<0.01
Patients without joint bleeds (%)	5	25		10	50	
Outcome						
Clinical score (max 90)	2 (0–5)	0 (0–4)	0.45	0 (0–2)	0 (0–0)	<0.01
Pettersson score (max 78)	10 (3.5–17.5)	4 (0–15)	0.75	0 (0–5)	0	<0.01

Values are medians (interquartile ranges). Reproduced from [16] with permission.

Recently, Schobess *et al*. [22] showed no differences in radiologic outcome at age 12.5 years when comparing 42 patients who started prophylaxis before the third bleed (including soft tissue bleeds) with 67 age-matched patients who started later. However, both groups started prophylaxis early: at a median age of 1.7 years and 2.5 years, respectively.

A practical strategy to start prophylaxis very early is to follow the example of Dr. Petrini and start with once-weekly infusions. This approach is more acceptable for parents and may reduce the need for central venous catheters. After starting once-weekly infusions, the Swedish aim to increase the frequency of infusions to three times weekly or every other day as soon as possible [23]. Others advocate increasing frequency according to bleeding pattern, increasing the number of weekly infusions stepwise at the occurrence of each joint bleed or severe bleed until patients take prophylaxis three times a week or every other day. Formal evaluations of this treatment strategy are still lacking. The Canadian group have formalized a step-up regimen a prospective trial in boys with hemophilia A with factor VIII activity levels of less than 2 IU/dL and normal joints on physical examination and imaging, patients with three or more bleeds in one joint were excluded. Their approach is to start with once-weekly 50 IU/kg between the age of 1 and 2.5 years, and increase stepwise to 30 IU/kg twice weekly and 25 IU/kg every other day at the occurrence of four significant bleeds within a 3-month period or five bleeds in one joint independent of time. A report on the first 25 boys with a median follow-up of 4.1 years was recently published [24]. Thirteen boys (52%) escalated to step 2 after a median of 2.3 years, and four boys (16%) escalated to step 3. The average number of joint bleeds was 1.2 per year, but nine patients (36%) still developed target joints, suggesting that the step-up criteria may be too lenient.

The discussion on how early to start prophylaxis is still ongoing. Patients on prophylaxis still experience joint bleeds, even on high-dose regimens [4,11]. Starting prophylaxis very early must be balanced against problems in venous access. Using a more individualized regimen, the use of central venous catheters may be avoided. Additional support for starting prophylaxis early has been provided by the results of the CANAL study group, suggesting that early prophylaxis may help prevent inhibitor formation [25].

Prophylaxis in adults

The aim of prophylactic replacement therapy is to prevent bleeds and their consequences, especially hemophilic arthropathy. Most countries which start with early prophylaxis continue prophylactic treatment for adults, as recommended by the WHO/WFH [8]. However, starting prophylaxis in adolescents and adults has not been included in available guidelines.

To continue prophylactic replacement therapy that has been started in early childhood is only logical: joint bleeds in adults still induce damage [26]. The early reports from Sweden and the Netherlands have shown that prophylaxis started in adults prevents bleeds and slows arthropathy [4,5]. However, the combination of high costs and uncertainty about the effects of prophylaxis in patients with established arthropathy have rendered starting prophylaxis in older patients controversial. Recently, Tagliaferri *et al*. reported on 84 patients who started prophylaxis during adolescence (n = 30) or adulthood (n = 54) [27]. As expected, the mean number of joint bleeds was significantly reduced (from 32.4 per year to 3.3 per year; $P < 0.01$), with a significant reduction in days lost from school or work, hospital visits, and hospital admissions.

Concomitantly, physical examination scores tended to improve (P = 0.13) and health-related quality of life improved significantly. Mean clotting factor consumption increased with less than 50% from 2871 to 3987 IU/kg/year. The lack of consensus on the benefits of continuous prophylaxis in adult patients is reflected by two surveys: one from Europe, reporting that only 23% of patients over 50 years old were on prophylaxis [28], and another from Canada, where 70 of 162 patients (43%) aged 31–70 years were on prophylaxis [29].

In contrast, it has been observed that some patients who have been treated with early prophylaxis consider discontinuing in adulthood. At entering the third decade of life, patients may get a job, and physical activity may be more confined to well-defined periods during the week. In the Netherlands, where primary prophylactic treatment is standard for all patients with severe hemophilia, about two-thirds of patients experiment with discontinuing their long-term prophylaxis. In their early 20s (median age 21.4 years), 35% of a cohort of 80 Dutch and Danish patients switched to on-demand treatment for more than 1 year [30]. It must be emphasized that patients discontinued on their own accord, without consulting their doctor. Having received early prophylaxis, these patients only experienced a median of three joint bleeds per year without prophylaxis. Median clinical scores were similar in patients who discontinued prophylaxis and those who continued, as were median Pettersson scores (median 13 points). However, longer evaluation is needed to assess the safety of discontinuing prophylaxis in these young adults. Later, this observation was confirmed by a European survey [28]: of 218 patients with severe hemophilia treated with early prophylaxis, 27% reduced the intensity of prophylaxis, and 42% discontinued prophylaxis. Eventually, 22% remained on reduced-intensity prophylaxis, and 30% continued on-demand treatment.

It is important to note that patients who switch to, and remain on, on-demand treatment are a subgroup with a milder bleeding pattern [31]. Currently, these patients may be best defined by a later onset of joint bleeding and a lower treatment requirement while on prophylaxis.

Prophylaxis versus on-demand therapy: issues of cost-effectiveness

Results of the most important published studies comparing prophylactic with on-demand treatment are shown in Table 5.3. Because it has been repeatedly demonstrated that clotting factor consumption accounts for over 90% of treatment cost in hemophilia [32,33], most studies have used clotting factor consumption as the main parameter for treatment cost. Three studies have been performed in children, and three in adults. Follow-up varied from 1 to 11 years. Selection bias was excluded by using an RCT in children, and reduced by comparing complete cohorts in two adult studies [34,35]. Only the study by Steen Carlsson was aimed at studying costs and benefits of prophylaxis.

All studies comparing prophylactic with on-demand treatment that are summarized in Table 5.3 consistently reported that prophylaxis was associated with fewer joint bleeds. Concomitantly, a 100–200% higher clotting factor consumption for patients on prophylaxis was reported in all but one study. The similar clotting factor consumption for both regimens reported by Fischer *et al.* [34] may be explained by lower clotting factor consumption on the Dutch intermediate dose regimen [16], together with the need for short courses of prophylactic treatment in the French cohort. From a methodologic point of view, it is important to avoid selection bias and include both patients with a milder and more severe bleed-

Table 5.3 Outcome and costs reported in studies comparing on-demand treatment (OD) with prophylaxis (PR).

Study	Design	Patients (*n*) (OD/PR)	Follow-up (years)	Age (years) (OD/PR)	Joint bleeds/ year (OD/PR)	Consumption (IU/kg/year) (OD/PR)	Orthopedic surgery (%) (OD/PR)
Children							
Aledort *et al.* [42]	Prospective cohort	411/66	5	13	16.5 vs. 5.7	1038 vs. 2772	NA
Smith *et al.* [32]	Retrospective cohort	90/27	2	7*	NA	1015 vs. 3323*	NA
Manco-Johnson *et al.* [11]	Prospective RCT	33/32	4	6	4.9 vs. 0.6	1819 vs. 5770#	NA
Adults							
Schramm *et al.* [43]	Retrospective cohort	670/335	1	35	7.7 vs. 3.4	1224 vs. 3208	–
Fischer *et al.* [34]	Retrospective complete cohort	103/49	1	22	16.9 vs. 5.1	1488 vs. 1612	20% vs. 55%
Steen Carlsson *et al.* [35]	Retrospective complete cohort	61/95	11	>18	NA	780 vs. 3024	9% vs. 52%

Values are given as means.

NA, not applicable; RCT, randomized controlled trial.

*Median values.

#Patients in both arms received enhanced treatment (total 80 IU/kg) for each joint bleed.

ing pattern for both treatment strategies [13]. Moreover, a state-of-the-art economic evaluation should present data on both short- and long-term outcome, and analyze from a societal perspective, including a cost–utility analysis [36]. Studies reporting cost per bleed avoided only will underestimate the cost-effectiveness of prophylaxis, as long-term benefits are not taken into account. Likewise, a cost–utility analysis should include lifetime data on treatment and outcome, rather than considering only 1 year of follow-up. Including all costs from a societal perspective with a follow-up of 11 years, Steen Carlsson reported that prophylaxis was still considerably more expensive than on-demand treatment. And even if people in Sweden were willing to pay for this treatment [37], this may change when respondents are forced to choose between different healthcare interventions. However, because prophylaxis clearly offers substantial clinical, and probably also psychological, benefits, this treatment strategy may be warranted on medical rather than economic grounds.

Implementation of prophylaxis

Despite an increasing number of favorable reports since the 1970s, numerous recommendations, and a recent RCT, the introduction of prophylaxis on a large scale has been slow. Even in Europe, where the cost of prophylaxis is usually covered, the use of prophylaxis is still increasing, as was demonstrated in the second survey of the European Paediatric Network (PEDNET). This survey in 22 centers showed that the number of centers using prophylaxis in almost all (80–100%) of their severe patients had increased from 11 in 1998 to 15 centers in 2003. Moreover, prophylaxis was started earlier [38]. In the USA, the high cost of treatment has been especially limiting. However, the use of prophylaxis is increasing too: recent surveys suggest that 44–53% of all patients with severe hemophilia were receiving prophylaxis in 2003 to 2005 [39,40]. In Canada, uptake has been earlier, and in 2006 69% of all patients with severe hemophilia A were on prophylaxis [29]. Outside Europe and the USA, treatment of hemophilia is still under development. Initiatives such as twinning provide important incentives to improve and intensify treatment in countries where clotting factor products are available [41].

Future issues in prophylaxis

Now that the positive effects of primary prophylaxis in young boys have been confirmed by the RCT by Manco-Johnson *et al.*, [11] it is expected that the use of primary prophylaxis will increase in countries with available products. Intermediate-dose regimens may be effectively used in countries with limited resources.

As with primary prophylaxis, the use of prophylaxis for adult patients will be adopted by some, and questioned by others. Currently, there is increasing debate about the use of prophylaxis in patients with inhibitors, in order to prevent the development of target joints and arthropathy, not only during the vulnerable period before and during immune tolerance treatment, but also in patients who failed immune tolerance. However, bypassing agents are less effective than factor VIII or IX, and the results of prophylaxis with bypassing agents may not be as favorable as with normal concentrates in patients without inhibitors.

The development of clotting factor products with prolonged half-life will be an important step forward. Once these products have been developed and are available, the number of infusions per week needed to protect patients from bleeding will decrease. This is expected to significantly reduce the burden of treatment for patients and improve adherence.

Conclusion

It has been proven that early prophylactic therapy can prevent bleeds and arthropathy. However, because of its high costs, it is currently only attainable for a small proportion of children with severe hemophilia. Because primary (early) prophylaxis is most effective in preventing arthropathy, this should be the treatment of choice for young children. The aim is to continue prophylaxis in adulthood; however, some patients with milder bleeding patterns may be able to reduce the intensity of their regimen. Prophylaxis is a very demanding lifelong treatment. The issues of venous access and frequent infusions may improve considerably after the introduction of clotting factor products with prolonged half-life.

References

1 Ahlberg A. Haemophilia in Sweden VII. Incidence, treatment and prophylaxis of arthropathy and other musculo-skeletal manifestations of haemophilia A and B. *Acta Orthop Scand* 1965; **77**(Suppl.): 5–99.
2 Nilsson IM, Blomback M, Ahlberg A. Our experience in Sweden with prophylaxis on haemophilia. *Bibl Haematol* 1970; **34**: 111–24.
3 Van Creveld S. Prophylaxis of joint hemorrhages in hemophilia. *Acta Haematol* 1969; **41**:206–14.
4 Löfqvist T, Nilsson IM, Berntorp E, Pettersson H. Haemophilia prophylaxis in young patients—a long-term follow-up. *J Intern Med* 1997; **241**: 395–400.
5 Fischer K, Van der Bom JG, Mauser-Bunschoten EP, *et al.* Changes in treatment strategies for severe haemophilia over the last 3 decades: Effects on clotting factor consumption and arthropathy. *Haemophilia* 2001; **7**: 446–52.
6 Ljung R, Aronis-Vournas S, Kurnik-Auberger K, *et al.* Treatment of children with haemophilia in Europe: A survey of 20 centres in 16 countries. *Haemophilia* 2000; **6**: 619–24.
7 National Hemophilia Foundation. MASAC recommendations concerning prophylaxis (regular administration of clotting factor concentrate to prevent bleeding). Medical Bulletin #193. Chapter Advisory 197. Available at: www.hemophilia.org/NHFWeb/MainPgs/MainNHF.aspx?menuid=57&contentid=1007. 3–11–1994

8 Berntorp E, Astermark J, Bjorkman S, *et al.* Consensus perspectives on prophylactic therapy for haemophilia: Summary statement. *Haemophilia* 2003; **9**(Suppl. 1):1–4.

9 Stobart K, Iorio A, Wu JK. Clotting factor concentrates given to prevent bleeding and bleeding-related complications in people with haemophilia A or B (review). *Cochrane Database Syst Rev* 2006; (2): CD003429.

10 White GC. Parachutes and prophylaxis: They both work! *J Thromb Haemost* 2006; **4**: 1226–7.

11 Manco-Johnson MJ, Abshire TC, Shapiro AD, *et al.* Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; **357**: 535–44.

12 Gringeri A, Lundin B, Von Mackensen S, Mantovani L, Mannucci PM. A 10-year, randomized, clinical trial on prophylaxis vs. on-demand treatment in children with haemophilia A: The E.S.P.R.I.T. study (Abstract). *Haemophilia* 2008; **14**(Suppl. 2): 99.

13 Fischer K, Grobbee DE, Van den Berg HM. RCTs and observational studies to determine the effects of prophylaxis in severe haemophilia. *Haemophilia* 2007; **13**: 345–50.

14 Matucci M, Messori A, Donati-Cori G, *et al.* Kinetic evaluation of four Factor VIII concentrates by model-independent methods. *Scand J Haematol* 1985; **34**: 22–8.

15 Carlsson M, Berntorp E, Bjorkman S, Lindvall K. Pharmacokinetic dosing in prophylactic treatment of hemophilia A. *Eur J Haematol* 1993; **51**: 247–52.

16 Fischer K, Astermark J, Van der Bom JG, *et al.* Prophylactic treatment for severe haemophilia: Comparison of an intermediate dose regimen to a high dose regimen. *Haemophilia* 2002; **8**: 753–60.

17 Astermark J, Petrini P, Tengborn L, Schulman S, Ljung RCR, Berntorp E. Primary prophylaxis in severe haemophilia should be started at an early age but can be individualized. *Br J Haematol* 1999; **105**: 1109–13.

18 Fischer K, Van der Bom JG, Mauser-Bunschoten EP, *et al.* Effects of postponing prophylactic treatment on long-term outcome in patients with severe haemophilia. *Blood* 2002; **99**: 2337–41.

19 Pollmann H, Richter H, Ringkamp H, Jürgens H. When are children diagnosed as having severe haemophilia and when do they start to bleed? A 10-year single-centre PUP study. *Eur J Pediatr* 1999; **158**: 166–70.

20 Van Dijk K, Fischer K, Van der Bom JG, Grobbee DE, Van den Berg HM. Variability in clinical phenotype of severe haemophilia: the role of the first joint bleed. *Haemophilia* 2005; **11**: 438–43.

21 Kreuz W, Escuriola-Ettingshausen C, Funk M, Schmidt H, Kornhuber B. When should prophylactic treatment in patients with haemophilia A and B start?—The German experience. *Haemophilia* 1998; **4**: 413–17.

22 Schobess R, Kurnik K, Friedrichs F, *et al.* Effects of primary and secondary prophylaxis on the clinical expression of joint damage in children with severe haemophilia A. Results of a multicenter non-concurrent cohort study. *Thromb Haemost* 2008; **99**: 71–6.

23 Petrini P. What factors should influence the dosage and interval of prophylactic treatment in patients with severe haemophilia A and B? *Haemophilia* 2001; **7**: 99–102.

24 Feldman BM, Pai M, Rivard GE, *et al.* Tailored prophylaxis in severe hemophilia A: Interim results from the first 5 years of the Canadian Hemophilia Primary Prophylaxis Study. *J Thromb Haemost* 2006; **4**: 1228–36.

25 Gouw SC, Van der Bom JG, Marijke van den BH. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: The CANAL cohort study. *Blood* 2007; **109**: 4648–54.

26 Jansen NW, Roosendaal G, Bijlsma JW, DeGroot J, Theobald M, Lafeber FP. Degenerated and healthy cartilage are equally vulnerable to blood-induced damage. *Ann Rheum Dis* 2008; **67**: 1468–73.

27 Tagliaferri A, Franchini M, Coppola A, *et al.* Effects of secondary prophylaxis started in adolescent and adult haemophiliacs. *Haemophilia* 2008; **14**: 945–51.

28 Richards M, Altisent C, Batorova A, *et al.* Should prophylaxis be used in adolescent and adult patients with severe haemophilia? An European survey of practice and outcome data. *Haemophilia* 2007; **13**: 473–9.

29 Biss TT, Chan AK, Blanchette VS, Iwenofu LN, McLimont M, Carcao MD. The use of prophylaxis in 2663 children and adults with haemophilia: Results of the 2006 Canadian national haemophilia prophylaxis survey. *Haemophilia* 2008; **14**: 923–30.

30 van Dij K, Fischer K, Van der Bom JG, Scheibel E, Ingerslev J, Van den Berg HM. Can long-term prophylaxis for severe haemophilia be stopped in adulthood? Results from Denmark and the Netherlands. *Br J Haematol* 2005; **130**: 107–12.

31 Fischer K, Van der Bom JG, Prejs R, *et al.* Discontinuation of prophylactic therapy in severe haemophilia: Incidence and effects on outcome. *Haemophilia* 2001; **7**: 544–50.

32 Smith PS, Teutsch SM, Shaffer PA, Rolka H, Evatt B. Episodic versus prophylactic infusions for hemophilia A: A cost-effectiveness analysis. *J Pediatr* 1996; **129**: 424–31.

33 Molho P, Rolland N, Lebrun T, *et al.* Epidemiological survey of the orthopedic status of severe haemophilia A and B patients in France. *Haemophilia* 2000; **6**: 23–32.

34 Fischer K, Van der Bom JG, Molho P, *et al.* Prophylactic versus on demand strategies for severe haemophilia: A comparison of costs and long-term outcome. *Haemophilia* 2002; **8**: 745–52.

35 Steen Carlsson K, Hojgard S, Glomstein A, *et al.* On-demand vs. prophylactic treatment for severe haemophilia in Norway and Sweden: Differences in treatment characteristics and outcome. *Haemophilia* 2003; **9**: 555–66.

36 Nicholson A, Berger K, Bohn R, *et al.* Recommendations for reporting economic evaluations of haemophilia prophylaxis: A nominal groups consensus statement on behalf of the Economics Expert Working Group of The International Prophylaxis Study Group. *Haemophilia* 2008; **14**: 127–32.

37 Steen Carlsson K, Hojgard S, Lethagen S, Lindgren A, Berntorp E, Lindgren B. Willingness to pay for on-demand and prophylactic treatment for severe haemophilia in Sweden. *Haemophilia* 2004; **10**: 527–41.

38 Chambost H, Ljung R. Changing pattern of care of boys with haemophilia in western European centres. *Haemophilia* 2005; **11**: 92–9.

39 Soucie JM, Cianfrini C, Janco RL, *et al.* Joint range-of-motion limitations among young males with hemophilia: Prevalence and risk factors. *Blood* 2004; **103**: 2467–73.

40 Khair K, Lawrence K, Butler R, O'Shea E, Christie BA. Assessment of treatment practice patterns for severe hemophilia A: A global nurse perspective. *Acta Haematol* 2008; **119**: 115–23.

41 Poon MC, Luke KH. Haemophilia care in China: Achievements of a decade of World Federation of Hemophilia treatment centre twinning activities. *Haemophilia* 2008; **14**: 879–88.

42 Aledort LM, Haschmeyer RH, Pettersson H. A longitudinal study of orthopaedic outcomes for severe factor-VIII-deficient haemophiliacs. The Orthopaedic Outcome Study Group. *J Intern Med* 1994; **236**: 391–9.

43 Schramm W, Royal S, Kroner B, *et al.* Clinical outcomes and resource utilization associated with haemophilia care in Europe. *Haemophilia* 2002; **8**: 33–43.

6 Continuous infusion of coagulation products in hemophilia

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Introduction

Continuous administration of coagulation factors at a rate corresponding to their pharmacokinetic elimination was originally suggested by Brinkhous in 1954 [1] and later supported by the mathematic model developed by Hermens for both factor VIII (FVIII) and factor IX (FIX) [2]. Hermens calculated that the dose needed to keep the plasma level above the hemostatic minimum increases with the interval between injections. The theoretical differences between continuous infusion (CI) and intermittent bolus injection (BI) are shown schematically in Figure 6.1. The area under the curve (AUC) corresponds with the total amount of FVIII required; thus, it can easily be seen that the AUC in CI is about one-third less compared with BI [3]. Hathaway *et al*. [4], in one of the first clinical observations with CI, could not confirm the expected 30% reduction in requirements by CI. Using a fixed rate of FVIII, he observed unexpected progressive increase of FVIII levels. His data, however, served as the ground for the development of the adjusted-dose CI [3,5,6]. We found that this elevation was the result of gradual decrease in the clearance after a steady hemostatic factor level has been achieved [3,5]. The reason for this phenomenon is still obscure; however, it provides a potential for additional saving of factor concentrates by adjustment of the rate of infusion proportionally to the decreasing clearance.

Extensive research conducted in the early 1990s addressed several important issues of CI treatment [3,5–7]. Extensive stability studies revealed that most concentrates at that time (but not all) were stable after reconstitution at room temperature for few days and more, in various minipumps [6]. The addition of minor amounts of heparin (5 U/mL) to the concentrates improved stability of some and prevented local thrombophlebitis at the site of infusion caused by the concentrated proteins. Inoculation of minipumps' reservoirs containing factor concentrates with common contaminants did not cause bacterial overgrowth and the thousands of cultures performed from minipumps' reservoirs after completion of the infusion were all negative [5–8]. A simple steady-state equation was adopted [5] to calculate the required rate of infusion and to adjust the rate to the decreasing clearance. Clinical trials have proven the efficacy and safety of CI, including at-home therapy settings [9] as well as the saving effect [8]. The guidelines for the administration of the adjusted-dose CI of coagulation factors introduced in 1992 [5] are still in use. Subsequently, many new-generation products have been proven to comply with the stability requirements for CI [10–13], and CI has become a widely used mode of factor replacement in many hemophilia centers worldwide.

Recently, an important issue arose from the observation of inhibitor development in several patients treated with an intensive treatment with CI [14–17]. However, the data are still inconclusive and prospective randomized multicenter studies are warranted to evaluate the risk of any intensive therapy employing either CI or BI regimes with respect to all potential confounding factors for this complication of hemophilia therapy.

Continuous infusion technique and stability of concentrates

An essential prerequisite for CI is factor concentrate maintaining its extended stability in the clinical settings of its use—i.e., after reconstitution, in the reservoir of the infusion pump at room temperature, with addition of minor amounts of heparin required to prevent local phlebitis at the site of infusion. The studies in the early 1990s demonstrated that most plasma-derived concentrates (pd-FVIII/FIX) available at that time remained stable for several days after their reconstitution, and sometimes even for weeks [5,6]. A good stability and low volume of concentrates allow to employ comfortable portable minipumps with the exchange of infusion bags with concentrated products at intervals of 1–3 days (and longer), making this treatment convenient [5]. Nevertheless, in the USA and Canada, 59% and 80%, hemophilia centers employing the CI method, respectively, still further dilute the concentrates with 60–500 mL of normal saline, and only 41% of US centers use

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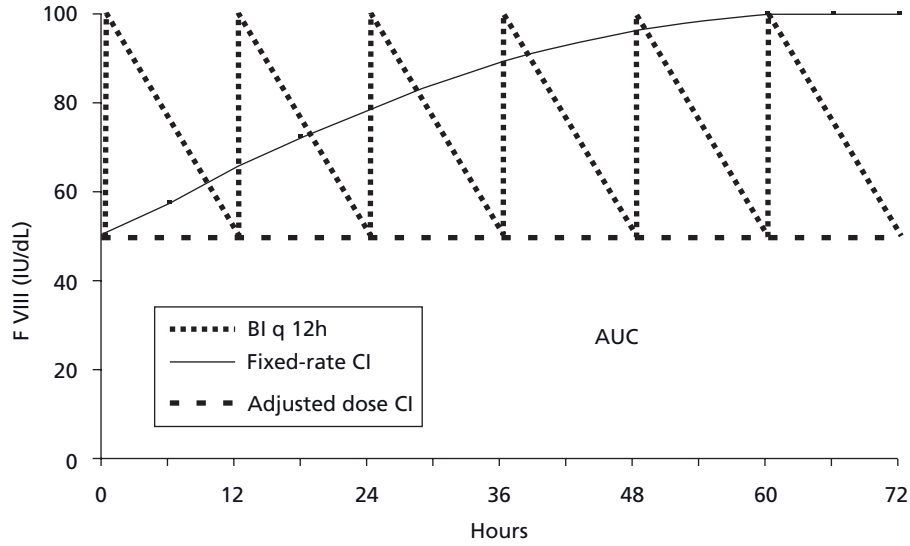

Figure 6.1 Factor VIII levels obtained during replacement with intermittent bolus injections (BI) and continuous infusion (CI) of fixed or adjusted doses. Reproduced from [3] with permission.

the products up to 24 h after reconstitution [16,18–21]. McLeod *et al*. [18] demonstrated that dilution of recombinant FVIII to 2–10 IU/mL causes considerable loss of FVIII activity (Figure 6.2). Proteolytic degradation during the storage was not found and the loss of activity was attributed to the adsorption of FVIII to the wall of container and tubing. Another example of instability of one pd-FVIII after reconstitution (progressive FVIII activation during the storage and significant decrease of FVIII activity after heparin addition) is shown in Figure 6.3. This resulted in the product withdrawal by the manufacturer (U. Martinowitz and S. Schulman, The National Hemophilia Center, Israel, unpublished). The contradictory reports on the influence of different material of infusion sets (polyvinylchloride, polyethylene, and polypropylene) on factor activity [10,18] were resolved by more recent experiments with simulated CI employing various infusion pumps. These experiments proved a good stability of all new-generation products tested, including recombinant FIX (rFIX) and FVIII (rFVIII) concentrates, which was influenced neither by the infusion set employed nor by heparin added in final concentration of 1–5 U/mL [11–13,22–25]. Nevertheless, before initiating CI it is advisable to test the stability of each concentrate type and to assay its compatibility with the particular infusion set to be used for CI.

Modern methods of hemophilia treatment augmented the requirements for the quality of products. An expanding use of CI emphasizes the manufacturers to prove stability of concentrates and their compatibility with the devices commonly employed in CI procedures. So far, only one product has been approved for CI by the regulatory agency [23].

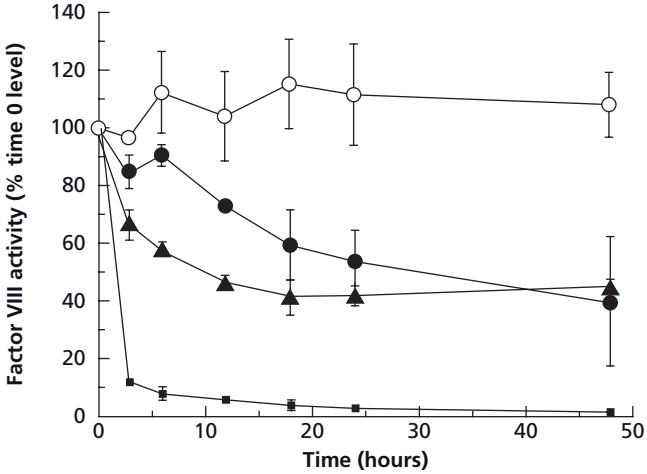

Figure 6.2 Effect of type of reservoirs and dilutions on FVIII activity: reconstituted undiluted product 146 IU/mL) in polypropylene syringes (○) or PVC mini-bags (●); product diluted to the concentration of 10 IU/mL (▲) and 2 IU/mL (■). Factor levels are expressed as a percentage of the initial factor FVIII activity (time 0 samples). Reproduced from [18] with permission.

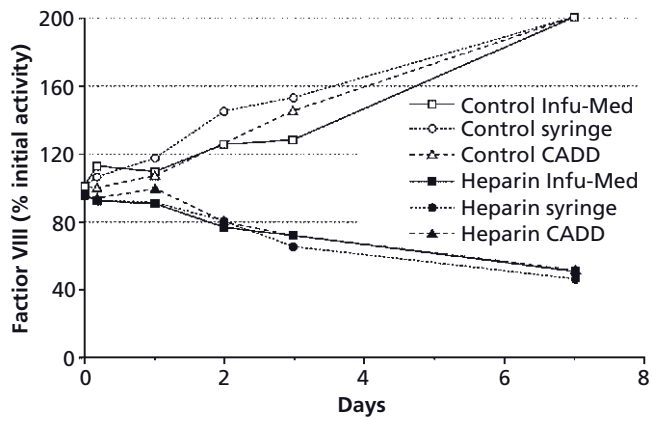

Figure 6.3 Batch #35002219. Stability of new pdFVIII: Progressive activation of FVIII observed in three different minipumps after reconstitution. Addition of minor amounts of heparin caused progressive reduction in FVIII activity. The product was withdrawn by the manufacturer (U. Martinowitz and S. Schulman, unpublished).

Bacteriologic safety of continuous infusion

The experiments focused on the risk of contamination and bacterial overgrowth during prolonged incubation of concentrates in the pump reservoir proved that many FVIII and FIX concentrates are poor growth mediums for most bacterial strains [3,5,6]. Studies employing simulated CI with minipumps failed to find any microbial contamination of the infusion sets used for up to 6–7 days [5,22], and the bacterial safety of CI is also confirmed by clinical trials [8,13]. To date, no clinical report has indicated an increased incidence of infectious complications after CI using peripheral vein access. In one report, central venous catheter infection impacted the clearance of administered FVIII [24]. This underscores a careful preparation and filling of the containers for CI under sterile conditions, e.g., under laminar air flow.

Prevention of thrombophlebitis

Thrombophlebitis at the site of venous access is a frequent adverse event of CI of undiluted FVIII concentrates, probably because of their high osmolarity [3]. This complication can be avoided by adding small amounts of heparin (5 U/mL) to the concentrate [3,5]. Such minor amounts of unfractionated heparin (UH) and low-molecular-weight heparin (LMWH) neither influence the stability of the reconstituted FVIII and FIX [5,22,25] nor affect *in vivo* hemostasis achieved by CI. In some products, addition of UH and LMWH may even improve stability, as in the case of rFVIII-FS [13]. In contrast, the addition of UH to rFVIIa leads to immediate loss of activity of 20–30%, and the addition of LMWH to rFVIIa causes aggregate formation without the loss of activity [26,27]. Although some centers still add LMWH to rFVIIa without diminishing the efficacy of CI, the most widely adopted method of preventing local phlebitis during CI of rFVIIa is the parallel infusion of saline (10–20 mL/h) through a three-way connector [27,28].

Modes of continuous infusion and treatment protocols

The dosing of factor replacement is based on both the clinical experience of the treating hematologist and specific pharmacologic calculations that take into account the basic pharmacokinetics of clotting factors, such as *in vivo* recovery and biologic half-life. The main goal is to achieve and maintain adequate hemostasis. However, although factor level is accepted by the regulatory agencies as a surrogate parameter for efficacy, the hemostatic minimum for particular clinical situations still remains to be empirical rather than evidence based. Continuous infusion is aimed at the maintenance of safe steady-state levels of the coagulation factor in circulation, eliminating unnecessary high peaks as well as trough levels which may fall below the minimum hemostatic level. A secondary, but no less important, goal of CI is to reduce the amount of factor required for the maintenance of desired hemostatic levels.

Adjusted-dose continuous infusion

This method employs pharmacokinetic dosing and takes advantage of decreasing clearance of coagulation factor during CI. The simple protocol for this method is based on the following set of principles [5]:

1 Pharmacokinetic evaluation prior to a planned CI is recommended but not mandatory. pharmacokinetic testing is based on a bolus administration of approximately 50 IU/kg of factor and measurement of factor levels before infusion and then at nine postinfusion time points over the following 36–48 h [29]. The most important pharmacokinetic parameter for calculating the ideal rate of continuous infusion is the clearance.

2 The loading dose is calculated using *in vivo* recovery (IU/dL per IU/kg). A dose is selected that will raise the level to the desired minimum level appropriate for the specific bleeding manifestation or surgical procedure requiring hemostatic replacement therapy.

3 CI is initiated immediately following bolus administration of the loading dose. The initial rate is calculated using the clearance obtained in the preprocedure pharmacokinetic evaluation according to the following steady-state equation:

$$\text{Rate of infusion (IU/kg/h)} = \text{clearance (mL/kg/h)} \times \text{desired level (IU/mL)}$$

4 From the second day, the CI maintenance dose is adjusted using the same equation according to actual clearance, which is calculated from the daily factor level measurements.

5 Acceptable target minimum FVIII levels for major surgery are 0.7–0.8 IU/mL, 0.5 IU/mL, and 0.3 IU/mL for postoperative days 1–3, 4–6, and 7–10, respectively.

6 Perioperative hemostatic demands may increase factor consumption beyond that expected. In order to prevent an undesired drop in the factor level, it is advisable to check factor activity, or at least the activated partial thromboplastin time (aPTT), 8–12 h after the start of CI, and to increase the rate if necessary.

7 In most patients who require treatment for more than 1 week, a significant decrease in FVIII clearance is observed during the first 5–6 days of CI, followed by a plateau at a significantly lower level than that observed in the first days postoperatively [5,8,30]. This allows one to reduce the maintenance dose progressively and results in a significant sparing of concentrate.

In the absence of preoperative pharmacokinetic evaluation or, in particular, in emergency situations, the initial maintenance dose may be calculated using the mean of a hemophilia population-based clearance, which is approximately 3.5 mL/kg/h for FVIII and 4.5 mL/kg/h for FIX. However, one has to be aware of possible interindividual variations in clearance, which may be influenced by age, body weight, laboratory assay employed, and even the type of factor concentrate used

[29,31,32]. Higher levels of clearance are physiologically observed in children.

Preoperative pharmacokinetic evaluation is a valuable tool to optimize CI, particularly if a major surgical procedure is planned. Pharmacokinetic testing may also alert one to an unsuspected low-titer inhibitor that may not be detectable by conventional inhibitor testing methods.

Fixed-rate continuous infusion

Some authors, aiming at FVIII/IX maintenance levels of 1.0 IU/mL for the first few postoperative days, have shown that this may be achieved with a fixed rate of FVIII and FIX of 4–5 IU/kg/h and ≥4 IU/kg/h, respectively, on average [16,17,21]. However, at this regimen often much higher levels are observed than those required to achieve satisfactory postoperative hemostasis [16,17,20,24]; further, during fixed-rate CI, the levels would be expected to rise gradually over the first 4–6 days owing to the decrease in clearance, which may result in unnecessarily high factor consumption unless appropriate adjustments are made daily, as described above.

Clinical indications for continuous infusion

Currently, the indications for continuous infusion of coagulation factors in hemophilia A and B are conditions that require the maintenance of efficient hemostatic factor levels for a prolonged period (longer than 3 days). Such situations include the treatment of major bleeds, minor and major surgical procedures, management of bleeding in some patients with low-titer or low-affinity inhibitors, and, rarely, short- and/or long-term prophylaxis. CI has been used also in home-therapy settings for large bleeds requiring the maintenance of adequate and sustained FVIII levels over several days [9], or after minor surgeries not requiring hospital admission.

Hemostatic safety and cost-efficacy of continuous infusion of factor VIII

Up to present, almost 100 reports comprising more than 1000 CI treatments reported CI to be safe and hemostatically effective for all indications mentioned above using either pdFVIII or rFVIII concentrates [3,5,8,16,17,20,33].

A prospective controlled study comparing CI and BI for major surgery using protocols that were similar regarding the surgical technique and postoperative target minimum levels demonstrated that efficacy and safety were better with CI than with BI in terms of minimum factor levels achieved, blood loss (as measured by a decrease in hemoglobin level), blood transfusion requirements, and bleeding complications (Table 6.1) [8]. The clearance of FVIII decreased over 6 days of CI from initial rates of 3.89 ± 0.86 mL/kg/h to a plateau at a minimum of 2.1 ± 0.54 mL/kg/h ($P < 0.01$). FVIII consumption was 36% lower in the CI group (total dose of FVIII for 13 days 467 ± 104 IU/kg vs. 733 ± 126 IU/kg; $P < 0.01$). A similar level of FVIII saving (30%) was observed also in a recent study comparing prospectively two groups of age- and procedure-matched patients treated with BI and CI (563.2 vs. 812.8 IU/kg; $P < 0.006$) [17]. Some centers seeking cost-effectiveness use low-dose CI protocols [34] while others emphasize safety rather than cost-effectiveness and use a high "fixed dose" instead of "adjusted dose" CI and target the steady-state levels around 1.0 IU/mL [16,17,20,24].

Table 6.1 Continuous infusion versus intermittent injections of factor VIII in severe hemophilia A patients undergoing major surgery [8].

	Bolus injections	Continuous infusion	*P*
Number (patients/operations)	18/18	22/25	
Age (years)	24 ± 14	26 ± 14	NS
Body weight (kg)	60 ± 17	58 ± 25	NS
Treatment period (days)	13 ± 1	13 ± 1	NS
FVIII levels[a]—first week (IU/mL)	0.43 ± 0.09	0.54 ± 0.09	<0.01
Nadir of FVIII—first week (IU/mL)	0.31 ± 0.09	0.44 ± 0.06	<0.01
Major bleeding complications	3/18	0/25	NS
Postoperative drop of hemoglobin (g/L)	30.1 ± 21.3	15.6 ± 12.1	<0.05
Patients requiring blood transfusion	7/18	3/25	<0.01
Factor consumption—first week (IU/kg)	493 ± 81	342 ± 69	<0.01
Total factor consumption (IU/kg)	733 ± 126	467 ± 104	<0.01

Values expressed as mean ± SD.
[a]Constant levels in continuous infusion and trough levels in bolus injection.

The comprehensive literature survey on CI practice demonstrated that the methods of CI of FVIII still vary from center to center in terms of initial infusion rates, steady-state factor levels aimed, reconstitution of products and infusion devices employed for CI [35]. Recent studies with CI of rFVIII products confirmed excellent efficacy of the method (Table 6.2); however, the differences in the study designs preclude to compare the outcomes regarding the final factor consumption.

Continuous infusion of factor IX

The stability studies and the first experience with CI of monoclonally purified FIX concentrate proved the feasibility of FIX

Table 6.2 Summary of recent reports on continuous infusion (CI) of recombinant FVIII in hemophilia A.

Study	Study design	Number of CI courses (number of patients)	CI duration (days) median*, mean ± SD (range)	Target levels days 1–5 (IU/mL)	Initial CI rate (IU/kg/h) median* or mean ± SD (range)	Product undiluted or diluted in 0.9% saline ± heparin, infusion device	Factor consumption (IU/kg/procedure) mean ± SD (range)
Dingli *et al.* [20]	R, SC	45 (28)	7* (2–51)	>1.0	4.0*	Diluted in 250ml saline	518
Stieltjes *et al.* [33]	P, MC	20 (16)	12 (5–35)	0.5–1.0	4.2* (1.8–6.0)	Undiluted 16 Diluted 4 UH added 8 Syringe pumps 6 Cassette pumps 14	791 (233–2249)
Mulcahy *et al.* [16]	R, SC	18 (12)	8.1 (2–20)	1.0	4.1 (2.4–5.0)	Diluted infusion	738 (36–3786)
Bidlingmaier *et al.* [17]	P, SC	43 (43)	7* (2–18)	0.8–1.0	4.4* (2.8–9.5)	Diluted Perfusor syringe pumps	Minor, n = 8 (465 ± 76) Medium, n = 24 (784 ± 115) Major, n = 11 (1057 ± 209)
Negrier *et al.* [24]	P, MC	18 (18)	NA	1.0	4.0–5.0	Undiluted Minipumps Syringe pumps	822 (401–2014)
Martinowitz *et al.* [13]	P, SC	15 (14)	9.2 ± 1,6	0.8	2.5–3.0	Undiluted LMWH added Minipump	426 ± 161

LMWH, low-molecular-weight heparin; MC, multicenter; NR, not reported; P, prospective; R, retrospective; SC, single center; UH, unfractionated heparin; undiluted, reconstituted without further dilution.

for this mode of factor replacement. However, with FIX it is also important to test the activation peptides to rule out the activation that may cause hypercoagulation [36,37]. Also, Hoots *et al.* [21], in a comprehensive prospective multicenter study, demonstrated the safety and hemostatic efficacy of CI of pd-FIX Mononine® in patients with hemophilia B undergoing surgery. Preoperative clearance was 4.06 mL/kg/h (median; range 2.45–9.65 mL/kg/h) and efficient hemostasis was achieved by median infusion rate of 3.84 IU/kg/h (range 1.74–7.33 IU/kg/h). Plasma-derived and recombinant FIX have different pharmacokinetics [32,38,39]. Poon *et al.* [38] observed *in vivo* recovery of 1.05 ± 0.26 IU/dL per IU/kg and 0.77 ± 0.19 IU/dL per IU/kg for pdFIX and rFIX, respectively, with no significant difference in half-life. In the CI setting the clearance of pdFIX may also differ significantly from that of rFIX (4.25 mL/kg/h vs. 7.71 mL/kg/h, respectively) [39]. To determine the clearance and to choose the dosing accordingly, pharmacokinetic evaluation prior to CI of FIX is recommended whenever possible [21].

Continuous infusion for long-term prophylaxis

Continuous infusion has potential for remarkable products saving in conditions of regular prophylaxis, as demonstrated by the theoretical calculations [40]; however, the method has still many limitations, mainly technical, for this indication. Nevertheless, CI was used for prolonged periods of 6–24 months in three of our patients after partial resection of a giant pseudotumor, in whom bleeding from the pseudotumor occurred when the FVIII level dropped below 10–20% [41]. The recent development of ultraconcentrated rFVIII with extended stability and development of tiny implantable minipumps might be promising, especially for the long-term prophylactic treatment.

Continuous infusion in patients with an inhibitor

Continuous infusion of factor VIII for the treatment of bleeding and induction of immune tolerance

Continuous infusion of human FVIII may be effective in the treatment of hemorrhage in patients with low-titer inhibitors and patients with low-affinity inhibitors such as in type II inhibitors in acquired hemophilia or in cases with partial success of immune tolerance induction. An immediate *in vivo* hemostasis may be achieved even when factor level remains undetectable. Shortening of the point-of-care aPTT suggests

that CI maintains factor levels sufficient to induce hemostasis. However, during the time from sampling through the centrifugation to testing in the lab the factor is neutralized in the test tube, resulting in immeasurable levels [3].

Intensive treatment with CI of FVIII administered in urgent situations resulted in the disappearance of inhibitor in several patients. Tamura *et al.* [42] observed such effect of CI administered postoperatively to a boy with a high-titer inhibitor at the infusion rate 6–12 IU/kg/h. High-dose CI of FVIII has also been introduced as a part of modified Malmö–Heidelberg protocol for inhibitor eradication in acquired hemophilia [43].

Continuous infusion of recombinant factor VIIa in hemophilia patients with inhibitors

Very short half-life of recombinant FVIIa (1.5–2.7 h) constitutes the rationale for CI of rFVIIa, which is aimed at saving the consumption of this expensive product. The method was used in the clinical situations shortly after the feasibility of rFVIIa for CI has been proven, and originally the target levels of FVII:C ≥10 U/mL were suggested [27]. However, these levels were demonstrated to be insufficient for major surgery [44]. Although the true level of FVII:C to maintain an effective hemostasis is still unknown, the growing clinical experience with rFVIIa and expanding knowledge on the mechanism of its action in the formation of a stable fibrin clot [45] subsequently also influenced the levels targeted in CI settings (Table 6.3). Plasma levels as high as 50 IU/mL and high CI maintenance doses around 50 μg/kg/h have been suggested and the clinical efficacy of such a high maintenance dose CI for major surgery was demonstrated [46,47]. Recently, the results of the first prospective multicenter randomized study comparing the BI and CI in inhibitor patients undergoing surgery were published [48]. After an initial dose of 90 μg/kg, 12 and 12 patients, respectively, undergoing surgery were treated with BI regime (90 μg/kg every 2 h on days 1–5 and 90 μg/kg every 4 h on days 6–10) and CI of rFVIIa (maintenance dose 50 μg/kg/h on days 1–5 and 25 μg/kg/h on days 6–10) [47]. The study demonstrated comparable hemostatic efficacy of rFVIIa in each group (9/12 and 8/11 patients treated with BI and CI, respectively). Higher mean postoperative levels of FVII:C in CI group compared with BI group (37.6 IU/mL vs. 25.9 IU/mL) were explained by a higher cumulative dose of rFVIIa in CI group (100 μg/kg per 2 h vs. 90 μg/kg per 2 h, respectively). The dose difference given by the treatment protocols preclude an objective comparison of total consumption of rFVIIa. All patients with the failure of treatment in this study had FVII:C levels >30 IU/mL at the time therapy was declared ineffective. This contrasts with the results of Santagostino *et al.* [28], who observed the hemostatic effect of CI of rFVIIa in 10/11 (91%) patients undergoing major surgery with median FVII:C levels of 25 IU/mL (13–70 IU/mL) using median maintenance dose of 20 μg/kg/h (15–50 μg/kg/h). These authors found no benefit of very-high-dose regimes (40–50 μg/kg/h) over maintenance doses of 15–20 μg/kg/h. CI of rFVIIa was also successfully used in patients with acquired hemophilia with similar efficacy and safety as the treatment with BI of rFVIIa [49].

Even though the high-dose CI regimes confer no cost benefit usually observed with CI, there can still be an improved standard of care by avoiding the inconvenience and mistakes arising from frequent intermittent injections.

Complications of continuous infusion

Most of the theoretical complications of CI—such as the instability of concentrates in the minipump and the pump failure, leading to unexpected drop in factor level below the hemostatic one, bacterial contamination of the reservoir, and frequent local phlebitis—can be prevented by meticulous planning before initiation of CI. This should include pharmacokinetic evaluation, the use of concentrates with known stability, preparation of infusion sets under sterile conditions,

Table 6.3 Summary of reports on continuous infusion (CI) of recombinant FVIIa for surgery.

Study	Study design	Number of CI courses (number of patients)	Infusion rate (μg/kg/h) median or mean[a] (range)	FVII:C (IU/mL) median or mean[a] (range)	Effective CI courses/total number of CI courses (%)
Smith *et al.* [44]	R, SC	6 (6)	16.5[a]	5—31[b] 6–16[c]	2/6 (33%)
Ludlam *et al.* [47]	R, SC	9 (9)	50	45 (17–88)[c]	9/9 (89%)
Santagostino *et al.* [28]	P, MC	11 major 14 minor	20 (15–50) 17 (6–24)	25 (13–70) 12 (6–24)	10/11 (91%) 13/14 (93%)
Pruthii *et al.* [48]	P, RD, MC	16 (12)	Days 1–5: 50 Days 6–10: 25	37.6[a]	9/12 (75%)[d]

MC, multicenter; P, prospective; R, retrospective; RD, randomized; SC, single center.

[a]Mean level; [b]levels on day 1; [c]levels on day 3; [d]number of patients with effective CI/number of patients treated with CI.

addition of a minor amount of heparin into the reconstituted concentrate, or use of the parallel infusion of saline to prevent phlebitis, as well as careful selection of a reliable infusion device.

Conflicting reports on inhibitor risk of CI occurred in the literature. Over the years, several new-onset inhibitor cases have been described with a major proportion of moderate/mild hemophiliacs in this group of patients having a low previous exposure to VIII [14–16]. In most cases, CI was given to patients in the context of extensive FVIII treatment, surgery and major bleedings, which are potential risk factors for inhibitor formation [50]. Patients who have undergone similar extents of FVIII exposure or surgical procedures have also developed inhibitors while using BI for administration [14,51]. Besides, an intensive treatment employing the high-dose CI protocols for mild/moderate hemophiliacs with low previous exposure to VIII may represent similar risk for inhibitor development as in previously untreated or minimally treated patients with severe hemophilia [14,17]. Therefore, the inhibitors may be attributed to these confounding factors and not to the method of administration. The type of gene mutation also may play a role, as shown by the most recent study of Eckhardt *et al*., demonstrating higher prevalence of Arg593→Cys mutation in moderate hemophilia patients developing inhibitors after intensive treatment for surgery [52].

Inhibitor formation after CI described in a small number of centers was not observed in centers with an extensive experience with CI. For instance, at the National Hemophilia Centers in Israel and Slovakia, more than 650 CI treatment courses for surgery and major bleeds have been performed in such patients without subsequent inhibitor development. This is supported also by a recent comprehensive review of the reports on cohort CI studies published in the literature, comprising 318 CI treatments in 258 hemophilia A patients, documenting inhibitors only in 5/258 (1.9%) patients treated with CI [35]. The most recent survey of current practice of CI in 22 European hemophilia centers demonstrated inhibitor in 10/868 (1.15%) patients who underwent 1072 CI treatments [53]. The first study prospectively focused on inhibitor incidence after CI for surgery did not reveal any inhibitor development in 46 patients with severe hemophilia A and B and von Willebrand disease [54]. Despite the arguments above against the connection of inhibitor risk and CI, the possible role of treatment conditions that promote neoantigenicity still remains open. The use of inappropriate CI technique, products unstable in an inappropriate infusion device and especially in large dilutions may probably result in conformational changes of FVIII with introduction of neoantigenicity. Therefore, only concentrates that are proven to be stable in the appropriate pumps used in the clinical setting should be approved for CI. Recombinant FVIII–Kogenate FS is currently the only EMEA-approved rFVIII preparation indicated for CI [13,23]. It is also crucial to employ exact dosing protocols which avoid overtreatment with undesirable high exposure to factor concentrates.

References

1 Brinkhous KM. Hemophilia. *Bull NY Acad Med* 1954; **30**: 325.
2 Hermens WTH. Dose calculation of human factor VIII and factor IX concentrates for infusion therapy. In: Brinkhou KM, Hemke HC (eds.) *Handbook of Hemophilia, Part II*. New York: American Elsevier Publishing, 1975: 569–89.
3 Martinowitz U, Schulman S. Continuous infusion of coagulation products. *Int J Pediatr Hematol/Oncol* 1994; **1**: 471–8.
4 Hathaway WE, Christian MJ, Clarke SL, Hasiba U. Comparison of continuous and intermittent factor VIII concentrate therapy in hemophilia A. *Am J Hematol* 1984; **17**: 85–88.
5 Martinowitz U, Schulman S, Gitel S, *et al*. Adjusted dose continuous infusion of factor VIII in patients with haemophilia A. *Br J Haematol* 1992; **82**: 729–34.
6 Schulman S, Gitel S, Martinowitz U. Stability of factor VIII concentrates after reconstitution. *Am J Hematol* 1994; **45**: 217–23.
7 Martinowitz U, Schulman S. Review of pumps for continuous infusion of coagulation factor concentrate: what are the options? *Blood Coag Fibrinolysis* 1996; **7**: 27–33.
8 Batorova A, Martinowitz U. Intermittent injections vs. continuous infusion of factor VIII in haemophilia patient undergoing major surgery. *Br J Haematol* 2000; **110**: 715–20.
9 Varon D, Schulman S, Beshari D, Martinowitz U. Home therapy with continuous infusion of factor VIII after minor surgery or serious haemorrhage. *Haemophilia* 1996; **2**: 207–10.
10 Hurst D, Zabor S, Malianni D, Miller D. Evaluation of recombinant factor VIII (Kogenate) stability for continuous infusion using a minipump infusion device. *Haemophilia* 1998; **4**: 785–9.
11 Neidhardt E, Koval R, Burke E, Wame N. In vitro evaluation of B-domain deleted recombinant factor VIII (ReFacto) stability during simulated continuous infusion administration. *Haemophilia* 2005; **11**: 319–25.
12 Fernandez M, Yu T, Bjornson E, *et al*. Stability of ADVATE, antihemophilic factor (recombinant) plasma albumin-free method, during simulated continuous infusion. *Blood Coag Fibrinol* 2006; **17**: 165–71.
13 Martinowitz U, Luboshitz, Bashari D. Stability, efficacy, and safety of continuously infused sucrose-formulated recombinant factor VIII (rFVIII-FS) during surgery in patients with severe hemophilia. *Haemophilia* 2009; **15**: 676–85.
14 Sharathkumar A, Lillicrap D, Blanchette S, *et al*. Intensive exposure to factor VIII is a risk factor for inhibitor development in mild haemophilia A. *J Thromb Haemost* 2003; **1**: 1228–36.
15 von Auer CH, Oldenburg J, von Depka M, *et al*. Inhibitor development in patients with hemophilia A after continuous infusion of FVIII concentrates. *Ann NY Acad Sci* 2005; **1051**: 498–505.
16 Mulcahy R, Walsh M, Scully MF. Retrospective audit of a continuous infusion protocol for haemophilia A at a single haemophilia treatment centre. *Haemophilia* 2005; **11**: 208–15.
17 Bidlingmaier C, Deml MM, Kurnik K. Continuous infusion of factor concentrates in children with haemophilia A in comparison with bolus injections. *Haemophilia* 2006; **12**: 212–17.
18 McLeod AG, Walker IR, Zheng S, Hayward CPM. Loss of factor VIII activity during storage in PVC containers due to adsorbtion. *Haemophilia* 2000; **6**: 89–92.
19 Osip J, Christie B, Hannemann V, Curoe S. Continuous infusion of factor concentrate practices in the United States—comparison of 1996 and 2005 surveys. *Haemophilia* 2006; **12**: 05PO111.
20 Dingli D, Gastineau DA, Gilchrist GS, *et al*. Continuous factor VIII infusion therapy in patients with haemophilia A undergoing

surgical procedures with plasma-derived or recombinant factor VIII concentrates. *Haemophilia* 2002; **8**: 629–34.
21 Hoots WK, Leissinger C, Stabler S, *et al.* Continuous infusion of a plasma derived factor IX concentrate (Mononine) in haemophilia B. *Haemophilia* 2003; **9**: 164–72.
22 Thomas KB, Urbancik W, Turecek PL, *et al.* Continuous infusion of FVIII and FIX concentrates: In vitro analysis of clinically relevant parameters. *Haemophilia* 1999; **5**: 17–25.
23 Framtpon JE, Wagstaff AJ. Sucrose—formulated octocog alfa: a review of its use in patients with haemophilia A. *Drugs* 2008; **68**: 839–53.
24 Négrier C, Shapiro A, Berntorp E, *et al.* Surgical evaluation of a recombinant factor VIII prepared using a plasma/albumin free method: Efficacy and safety of Advate in previously treated patients. *Thromb Haemost* 2008; **100**: 217–23.
25 Chowdary P, Dasani H, Jones JA, *et al.* Recombinant factor IX (BeneFix) by adjusted continuous infusion: a study of stability, sterility and clinical experience. *Haemophilia* 2001; **7**: 140–45.
26 Bonde C, Jensen M. Continuous infusion of recombinant activated factor VII: Stability in infusion pump system. *Blood Coagul Fibrinolysis* 1998; **9**: 103–5.
27 Schulman S for the rFVIIa-CI group. Continuous infusion of recombinant factor VIIa in haemophilic patients with inhibitor: Safety, monitoring, and cost effectiveness. *Semin Thromb Haemost* 2000; **26**: 421–4.
28 Santagostino E, Morfini M, Rocino A, *et al.* Relationship between factor VII activity and clinical efficacy of recombinant factor VIIa given by continuous infusion to patients with factor VIII inhibitors. *Thromb Haemost* 2001; **86**: 954–8.
29 Lee M, Morfini M, Négrier C, Chamouard V. The pharmacokinetics of coagulation factors. *Haemophilia* 2006; **12**(Suppl. 3): 1–7
30 Hay CRM, Doughty HI, Savidge GF. Continuous infusion FVIII for surgery and major bleeding. *Blood Coagul Fibrinolysis* 1996; **7**: 15–19.
31 Björkman S, Folkesson A, Berntorp E. In vivo recovery of factor VIII and factor IX: intra- and interindividual variance in a clinical setting. *Haemophilia* 2007; **13**: 2–8.
32 Ewenstein BM, Joist JH, Shapiro AD. Pharmacokinetic analysis of plasma-derived and recombinant FIX concentrates in previously treated patients with moderate or severe hemophilia B. *Transfusion* 2002; **42**: 190–7.
33 Stieltjes N, Altisent C, Auerswald G, *et al.* Continuous infusion of B-domain deleted recombinant factor VIII (ReFacto) in patients with haemophilia A undergoing surgery: Clinical experience. *Haemophilia* 2004; **10**: 452–8.
34 Srivastava A. Dose and response in haemophilia—optimization of factor replacement therapy. *Br J Haematol* 2004; **127**: 12–25.
35 Batorova A, Martinowitz U. Continuous infusion of coagulation factors: Current opinion. *Curr Opin Hematol* 2006; **13**: 308–15.
36 Schulman S, Gitel S, Zivelin A, *et al.* The feasibility of using concentrates containing factor IX for continuous infusion. *Haemophilia* 1995; **1**: 103–10.
37 Martinowitz U, Schulman S. Continuous infusion of factor concentrates: Review of use in hemophilia A and demonstration of safety and efficacy in hemophilia B. *Acta Haematol* 1995; **94**: 35–42.
38 Poon M-CH, Lillicrap D, Hensman C, *et al.* Recombinant factor IX recovery and inhibitor safety: A Canadian post-licensure surveillance study. *Thromb Haemost* 2002; **7**: 431–5.
39 Hermans C, Brown S, Harrington C, *et al.* Differences in clearance of plasma-derived versus recombinant factor VIII and IX during continuous infusion. *Thromb Haemost* 2001; (Suppl): OC1746.
40 Carlsson M, Berntorp E, Björkman S, Lindvall K. Pharmacokinetic dosing in prophylactic treatment of haemophilia A. *Eur J Haematol* 1993; **51**: 247–52.
41 Heim M, Martinowitz U. Part IX. Musculoskeletal: Pseudotumors in patients with hemophilia. In: Lee CA, Berntorp EE, Hoots WK (eds). *Textbook of Hemophilia*. Oxford: Blackwell Publishing, 2005: 174–6.
42 Tamura K, Kanazawa T, Suzuki M, *et al.* Successful induction of immune tolerance by continuous infusion of recombinant FVIII in a haemophilia A patient with high inhibitor titres. *Haemophilia* 2006; **12**: 100–2.
43 Huth-Kühne A, Ehrenfort S, Scharrer I, Zimmermann R. A new treatment option for patients with acquired haemophilia A. The modified Malmö–Heidelberg protocol. *Thromb Haemost* 2001; (Suppl.): CD3364, A.
44 Smith MP, Ludlam CA, Collins PW, *et al.* Elective surgery on factor VIII inhibitor patients using continuous infusion of recombinant activated factor VII. *Thromb Haemost* 2001; **86**: 949–53.
45 Hedner U. Treatment of patients with factor VIII and factor IX inhibitors with special focus on the use of recombinant factor VIIa. *Thromb Haemost* 1999; **26**: 102–8.
46 Tagariello G, Bisson R, Radossi P, *et al.* Concurrent total hip and knee replacements in a patient with haemophilia with inhibitors using recombinant factor VIIa by continuous infusion. *Haemophilia* 2003; **9**: 738–40.
47 Ludlam C, Smith M, Gringeri A, Savidge G. Pharmacokinetics of rFVIIa. *Haemophilia* 2002; **8**: 574.
48 Pruthi RK, Mathew P, Valentino LA, *et al.* Haemostatic efficacy and safety of bolus and continuous infusion of recombinant factor VIIa are comparable in haemophilia patients with inhibitors undergoing major surgery. Results from an open-label, randomized, multicenter trial. *Thromb Haemost* 2007; **98**: 726–32.
49 Baudo F, de Cataldo F, Gaidano G. Treatment of acquired factor VIII inhibitor with recombinant activated factor VIIa: Data from the Italian registry of acquired hemophilia. *Haematologica* 2004; **89**: 759–61.
50 Gouw SC, van der Bom JG, Auerswald G, *et al.* Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: The CANAL cohort study. *Blood* 2007; **109**: 4648–54.
51 Ghosh K, Jijina F, Shetty S, *et al.* First-time development of FVIII inhibitor in hemophilia patients during the postoperative period. *Haemophilia* 2002; **8**: 776–9.
52 Eckhardt CL, Menke LA, Ommen CH, *et al.* Intensive perioperative use of factor VIII and the Arg593→Cys mutation are risk factors for inhibitor development in mild/moderate haemophilia A. *J Thromb Haemost* 2009; **7**: 930–7.
53 Batorova A, Holme PA, Gringeri A, *et al.*, for the EHTSB. Continuous infusion practice in 22 haemophilia comprehensive care centres in 16 European countries. *J Thromb Haemost* 2009; **7**(Suppl. 2): PP-WE:656.
54 Auerswald G, Bade A, Hohne K, *et al.* Prospective study of inhibitor incidence with continuous infusion of factor concentrates during and after surgical procedures in patients with haemophilia A, B or von Willebrand disease. *J Thromb Haemost* 2009; **7**(Suppl. 2): PP-Mo-603.

7 Inhibitors to factor VIII—immunology

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Introduction

The immune response toward factor VIII (FVIII) presents several characteristics that make it unique. Antibodies to FVIII are made by healthy individuals, by patients suffering from hemophilia A, and by patients affected by some autoimmune diseases. FVIII is an autoantigen in the first and third of these situations. In the second instance, FVIII is administered intravenously and on a recurrent basis. The diverse characteristics make it essential to consider the immune response to FVIII from a general point of view, and not just as a peculiar response occurring in only a proportion of patients with hemophilia A.

The purpose of this chapter is to review our current understanding of the homeostasis of the anti-FVIII response, to summarize information recently gathered from animal models, and to update data obtained from relevant clinical observations.

Homeostasis of the anti-FVIII immune response

The production of antibodies to FVIII, analogous to the immune response to any soluble glycoprotein, depends on the interaction between specific B and T lymphocytes. The second circumstance in which T cells might not be required occurs when memory B cells are reactivated. The repertoire of T cells is established primarily in the thymus (see ref. 1 for a review). The role of the latter is threefold: (i) to eliminate T cells that do not recognize major histocompatibility complex (MHC) class I or class II determinants, a process through which CD4/CD8($^-$) T cells mature into $CD8^+$ or $CD4^+$ respectively; (ii) to eliminate T cells that recognize with high affinity the complex of self-epitopes and MHC determinants; and (iii) to select a population of regulatory T cells expressing CD25. For a number of reasons, it is unrealistic to expect that such a selection will eliminate all T cells with the capacity to react with FVIII.

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By contrast, the B-cell repertoire is continuously replenished over the lifespan [2]. Random rearrangement of the B-cell receptor (BCR) in the bone marrow generates cells with the potential of reacting with FVIII. The majority of autoreactive B cells are eliminated before entering the periphery. However, B cells use a number of mechanisms by which they can further diversify in the periphery. This is rendered possible by somatic hypermutation, which is a property of B cells. It involves the random introduction of mutations in antibody hypervariable regions, followed by affinity-driven selection.

All conditions are therefore assembled for an immune response to FVIII to emerge: specific T and B cells are present. However, the mechanisms by which the immune response is kept under control, i.e., without emergence of inhibitor antibodies, are many: Specific cells maintained in a state of anergy or unresponsiveness, the presence of anti-idiotypic antibodies, and regulatory T cells are but a few of these mechanisms. However, subtle alterations in this equilibrium can rapidly lead to the production of antibodies.

Lessons from animal models

Significant progress in our understanding of how anti-FVIII murine antibodies are elicited has been made since the mouse hemophilia A model became available. Strains with target disruption of exons 16 or 17 mimic the situation of severe hemophilia A patients and have been used to study the conditions under which antibodies are generated.

Injection of physiologic quantities of human rFVIII by the intravenous route elicits a strong antibody response, with T-cell activation observed after only 3 days [3]. The characteristics of the antibody response match those observed in patients with inhibitors, namely long-term persistence of significant antibody titers, dependence on costimulatory signals, and resistance to suppression of established responses [4]. An additional insight gleaned from FVIII immunization in hemophilia A mice is that von Willebrand factor (VWF) may somehow affect the immunogenicity of FVIII, both by reducing the overall antibody response toward FVIII and by modifying the profile of antibody specificity [5]. These results must be interpreted with caution, however, since human FVIII was used for these experiments. There is evidence that the

immunogenicity of mouse FVIII in such a model could be different, both qualitatively and quantitatively [6].

Not surprisingly, the mouse model of hemophilia A lends itself to the testing of various approaches of immunomodulation. Thus, information on how to suppress an immune response gleaned from other fields of immunologic research has been implemented in hemophilia A mice [3,7,8]. All these studies open therapeutic opportunities to prevent inhibitor formation, but fall short for the control on an ongoing inhibitor formation.

This was tackled by another research group, which demonstrated that administration of FVIII at doses equivalent to what would be given to a patient under immune tolerance therapy resulted in induction of apoptosis of FVIII-specific memory B cells [9]. This was indeed the first demonstration of the mechanism of action of a therapy (tolerance induction with FVIII) widely used for the last two decades. The question is, of course, whether the heterogeneous population of FVIII-reactive B cells (various specificities and affinities, various stages of maturation, etc.), which prevails in hemophilia A patients with inhibitors would be equally sensitive to such an approach.

Whether long-term prevention and suppression of inhibitor formation is feasible, under strict antigen-specific conditions, is suggested by studies showing that B cells engineered by transfection to present FVIII can induce both prevention and suppression of inhibitors [10].

Animal models of hemophilia will no doubt continue to shed light on the mechanisms controlling the immune response to FVIII, in addition to providing valuable alternative therapies for inhibitors. Humanized models, such as mice expressing MHC class II alleles or mice expressing transgenic FVIII receptors, should refine our understanding of such mechanisms.

Clinical observations

Characterization of anti-FVIII antibodies

Physicochemical characteristics

Antibody specificity

Mapping B-cell epitopes on FVIII has been the subject of many studies [11,12]. The presence of antibodies to FVIII is often confused with the presence of inhibitor antibodies, which constitute only a subset of antibodies with functional properties related essentially to the epitope they recognize. Whether or not noninhibitory antibodies can alter other parameters of the FVIII physiology is not entirely clear (see below, Mechanisms of FVIII inactivation).

To date, clusters of B-cell epitopes have been identified primarily on the C2 and A2 domains [11]. However, in the case of the C2 epitopes, it is known that the three-dimensional conformation is important for full antibody recognition, as shown by the importance of the disulfide bridge within this domain.

The diversity of antibodies recognizing a given cluster have recently been outlined by the characterization of a large collection of mouse monoclonal antibodies recognizing the FVIII C2 domain. Competition experiments between such antibodies and patient polyclonal antibodies demonstrated the existence of the different types of anti-C2 antibodies among patients' anti-FVIII antibodies [13].

Additional clusters of B-cell epitopes have been described on the A3 [14] and C1 [15] domains, the frequency of which is unclear. Immortalization of B lymphocytes as well as phage display will continue to provide information on antibodies directed to the A2, A3, C1, and C2 domains, and will help to determine the repertoire of immunoglobulin genes coding for anti-FVIII antibodies [16,17].

The first of such characterized human monoclonal antibodies, BO2C11, recognizes the FVIII C2 domain and inhibits FVIII binding to both phospholipids and VWF [17]. The antigenic determinant recognized by BO2C11 was determined by the crystallographic study of BO2C11 Fab fragments bound to recombinant C2 domain [18]. BO2C11 makes direct contacts with most hydrophobic and basic residues predicted to mediate FVIII binding to phospholipids, which is consistent with its inhibitory activity.

Antibody isotype and genetic origin

The anti-FVIII antibody response recruits all subclasses of immunoglobulin G (IgG), but the IgG4 isotype is somewhat over-represented [19], considering that IgG4 accounts for only 3% of the total IgG concentration in plasma. IgG4 is associated with long-term exposure to antigens [14], a situation that characterizes hemophilia A patients with longstanding inhibitors. In man, the isotype switch from IgM to IgG4 has been attributed to the presence of interleukin 4 (IL-4) and/or IL-13, the very cytokines involved in the production of IgE antibodies [15].

Yet IgE antibodies against FVIII are not observed, in contrast to what is occasionally observed in the case of FIX inhibitors. This paradox may, however, have been solved by recent data indicating that regulatory T cells can induce the production of IgG4 through mechanisms involving glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR)—GITR–ligand interactions, IL-10, and transforming growth factor (TGF)-b [20].

Functional properties

Kinetics of factor VIII inactivation

One usually distinguishes two types of inhibitor antibodies: Type 1 antibodies completely inhibit FVIII procoagulant activity following second-order kinetics, whereas type 2 antibodies follow more complex kinetics and cannot inhibit FVIII

completely [21]. The reason for such a difference is not known. It may be related to different mechanisms of FVIII inactivation (see below) and/or to differences in affinity, although it is possible that interaction with VWF plays a role. Indeed, Gawryl and Hoyer [22] reported that, for most type 2 inhibitors, FVIII inactivation was partial only when the antibody was in presence of VWF. The later observation that antibodies to the A3 and C2 domains competed with VWF for binding to FVIII provided an explanation for the effect of VWF on inhibitor kinetics. It is noteworthy that antibodies competing with VWF and with a sufficiently high affinity for FVIII can inactivate FVIII completely (type I inhibitor), albeit following a complex kinetics because binding to FVIII requires the preliminary dissociation of the FVIII–VWF complex [17]. Alternatively, VWF can be required for inhibitor activity. In such a case, it is likely that antibodies reduce the rate of dissociation of FVIIIa from VWF [23].

In contrast, rare antibodies only partially inhibit FVIII even in the absence of VWF [22,24]. The human monoclonal antibody LE2E9, which was derived from a patient with mild hemophilia A with inhibitor, recognizes the FVIII C1 domain and inhibits only 85% FVIII activity in the absence of VWF. The mechanism of action of this antibody is still under investigation, but its high affinity ($K_d = 0.5 \cdot 10^{-9}$ mol/L) indicates that, when it is present in excess over FVIII, all FVIII molecules must be complexed to the antibody [24]. Antibodies such as LE2E9, likely, therefore, reduce the cofactor activity of the FVIII molecule in the Xase complex.

Whatever the precise reason for this difference, the distinction between type 1 and type 2 inhibitors remains useful. Thus, type 1 inhibitors are most often observed in severe hemophilia A patients who respond to FVIII infusions by producing high antibody titers. In contrast, type 2 inhibitors are observed preferentially in mild or moderate hemophilia A patients, in previously untreated patients (PUPs) mounting a transitory response to FVIII infusion, and in patients producing antibodies toward FVIII molecules altered by preparation procedures [25]. This distinction is also relevant for the bleeding phenotype and response to treatment. Type 2 inhibitor patients usually present with skin and soft tissue bleeding rather than the joint and intraorgan bleeding observed in patients with type 1 inhibitors. In addition, eradication of the inhibitor, either spontaneously or as the result of infusion with high doses of FVIII, is readily achieved in patients with type 2 inhibitors, whereas type 1 inhibitor patients are far less responsive [26].

Mechanisms of factor VIII inactivation

One usually distinguishes two main categories of inhibitor antibodies. In the first case, antibodies bind to or within short distance of a site of FVIII that is involved in its function. It is worth noting that almost all possibilities have been illustrated by the study of mouse or human anti-FVIII antibodies. Thus, antibodies have been observed that inhibit the binding of FVIII to phospholipid, to VWF [11], to FIXa [11,27], and to thrombin or FXa [28], thereby interfering with thrombin cleavage. On the other hand, binding to FVIII sites involved in inactivation, essentially by FXa or activated protein C (aPC), have also been described [29], although the clinical relevance of such antibodies is less well understood.

In addition to this generic steric hindrance mechanism, antibodies can be formed to epitopes that are accessible only when the molecule is either bound to VWF or activated. Such antibodies are much more difficult to distinguish from polyclonal antibody populations, making it difficult to determine either their prevalence and/or their clinical relevance [23].

Recently, antibodies with catalytic activity to FVIII have been described, demonstrating a strong correlation between such an activity and the titer of inhibitor antibodies. Their presence is detectable in ~50% of hemophilia A patients with inhibitors [30]. Highly purified preparations of polyclonal anti-FVIII antibodies exert this catalytic activity when contaminating enzymes are excluded.

Notably, the majority of anti-FVIII antibodies do not interfere with FVIII function, as evaluated by current assay systems [12]. However, it is not yet clear whether or not such "non-functional" antibodies have a pathophysiologic role. It has been suggested that such antibodies could increase the clearance rate of FVIII from the circulation [31], perhaps thereby increasing the uptake of FVIII–immunoglobulin complexes by phagocytic cells of the reticuloendothelial system.

Factor VIII-specific T cells

Several clinical observations indicate that FVIII-specific T cells support the development of the humoral response to FVIII. In some patients with an established humoral response to FVIII, human immunodeficiency virus (HIV) infection leads to a decline in FVIII inhibitor as well as T-cell counts [32]. A large proportion of anti-FVIII antibodies belong to the IgG4 subclass [19]. This pinpoints a role for T cells in the development of the humoral response to FVIII since isotype switching is T-cell dependent. Lastly, hypermutations have been consistently detected in the genes coding for the variable part of cloned anti-FVIII antibodies [16,17]. This indicates that B cells secreting anti-FVIII antibodies undergo affinity maturation processes that require specific T-cell help. The recent observation that polymorphisms in the T-cell *CTLA-4* gene influence the incidence of inhibitor development [33] further supports a role of T cells in inhibitor production.

Factor VIII-specific T cells have been identified in the peripheral blood of hemophilia A patients with inhibitor using T-cell proliferation assays with native FVIII [34]. The epitope(s) recognized by such T cells have been mapped using synthetic peptides covering the entire FVIII molecule [35]. In severe hemophilia A patients, FVIII-specific T cells recognizing a large array of peptides scattered over the entire FVIII molecule have been detected. T cells proliferating in response to such peptides have also been identified in hemophilia A patients

without an inhibitor and in healthy individuals. Only minor qualitative or quantitative differences have been identified between FVIII-specific T cells isolated from healthy individuals and T cells isolated from hemophilia A patients with or without inhibitor.

However, clearcut differences between normal individuals and hemophilia A patients with an inhibitor have been observed when FVIII-specific T cells were examined at the clonal level. FVIII-specific T-cell lines were expanded and cloned using dendritic cells and FVIII-specific lymphoblastoid cells loaded with native FVIII. Under these experimental conditions, successful isolation of FVIII-specific T-cell lines was reported when blood from a hemophilia A patient with inhibitor was used but not when blood of normal individuals was used (ref. 39 and Renaud Lavend'homme, University of Leuven, personal communication). The epitope specificity of the few T-cell lines characterized so far is also much more restricted than that observed when peptides are used to stimulate $CD4^+$ T cells isolated from blood of hemophilia A patients or from normal individuals [36,37].

Such seemingly discrepant observations reiterate what was observed from animal studies and clinical observations in other fields such as autoimmune diseases (see above, Homeostasis of the anti-FVIII immune response). Two populations of FVIII-specific T cells coexist in the periphery. One population is detectable in normal individuals and in hemophilia A patients irrespective of inhibitor status and is demonstrable only when the entire FVIII-specific T-cell repertoire is screened through the use of peptides. A second population of bona fide pathogenic T cells is present only in patients with inhibitor antibodies.

Factor VIII is present in relatively low concentrations in plasma (0.2 µg/ml, 1 nM), which raises the question of how FVIII can be presented by professional antigen-presenting cells to T cells. Capture of FVIII by human dendritic cells is mediated at least in part by macrophage mannose receptor (CD206) expressed on dendritic cells by mannose-terminating glycans on FVIII [38]. However, the interaction between FVIII and CD206 is blocked by VWF [39] so that *in vivo* it cannot be excluded that other mechanisms could be exploited by antigen-presenting cells to present FVIII to T cells.

Perspectives

Many questions remain concerning the immune response to FVIII. However, it is clearly worth pursuing these types of investigations. Beyond the primary goal, which is to provide new methods to prevent and/or suppress the production of inhibitors in patients, more broadly applicable information will undoubtedly be gathered.

The anti-FVIII immune response represents the only known situation in which allo- and autoimmune responses are observed concurrently and in which patients at risk can be followed longitudinally. This offers the possibility of studying the way in which tolerance is established in central and peripheral organs. With the help of suitable animal models and experiments carried out at the clonal level (including transgenic animals), there is little doubt that mechanisms leading to anti-FVIII immune responses will be progressively unraveled.

References

1 Walker LS, Abbas AK. The enemy within: Keeping self-reactive T cells at bay in the periphery. *Nature Rev Immunol* 2002; **2**: 11–19.

2 McHeyzer-Williams M. B cell as effectors. *Curr Opin Immunol* 2003; **15**: 354–61.

3 Qian J, Collins M, Sharpe AH, Hoyer LW. Prevention and treatment of factor VIII inhibitors in murine hemophilia A. *Blood* 2000; **95**: 1324–9.

4 Reipert BM, Ahmad RU, Turecek PL, Schwarz HP. Characterization of antibodies induced by human Factor VIII in a murine knockout model of hemophilia. *J Thromb Haemost* 2000; **84**: 826–32.

5 Behrmann M, Fasold H, Pasi J, *et al.* Von Willebrand factor modulates factor VIII immunogenicity: Comparative study of different plasma-derived and recombinant factor VIII concentrates in a hemophilia A mouse model. *Thromb Haemost* 2002; **88**: 2221–9.

6 Doering C, Parker ET, Healey JF, *et al.* Expression and characterization of recombinant murine factor VIII. *Thromb Haemost* 2002; **88**: 450–8.

7 Waters B, Qadura M, Burnett E, *et al.* Anti-CD3 prevents factor VIII inhibitor development in hemophilia A mice by a regulatory CD4 + CD25+-dependent mechanism and by shifting cytokine production to favour a Th1 response. *Blood* 2009; **113**: 193–203.

8 Peng B, Ye P, Blazar BR, Freeman GJ, et al. Transient blockade of the inducible costimulator pathway generates long-term tolerance to factor VIII after non-viral gene transfer into hemophilia A mice. *Blood* 2008; **112**: 1662–72.

9 Hausl C, Ahmad RU, Sasgary M, et al. High-dose factor VIII inhibits factor VIII-specific memory B cells in hemophilia A with factor VIII inhibitors. *Blood* 2005; **106**: 3415–22.

10 Lei TC, Scott DW. Induction of tolerance to factor VIII inhibitors by gene therapy with immunodominant A2 and C2 domains presented by B cells as Ig fusion proteins. *Blood* 2005; **105**: 4865–70.

11 Scandella DH. Properties of anti-factor VIII antibodies in haemophilia A patients. *Semin Thromb Haemost* 2000; **26**: 137–42.

12 Gilles JGG, Arnout J, Vermylen J, et al. Anti-factor VIII antibodies of haemophiliac patients are directed primarily towards non-functional determinants and do not exhibit isotypic restriction. *Blood* 1993; **82**: 2452–61.

13 Meeks SL, Healey JF, Parker ET, et al. Nonclassical anti-C2 domain antibodies are present in patients with factor VIII inhibitors. *Blood* 2008; **112**: 1151–3.

14 Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology* 2002; **105**: 9–19.

15 Vercelli D. One cytokine, two isotypes. A Trojan horse, Pandora's box and evolving paradigm. *Am J Respir Crit Care Med* 2000; **162**: S86–S90.

16 van den Brink EN, Bril WS, Turenhout EA, et al. Two classes of germline genes both derived from the V(H)1 family direct the formation of human antibodies that recognize distinct antigenic

sites in the C2 domain of factor VIII. *Blood* 2002; **99**: 2828–34.
17 Jacquemin MG, Desqueper BG, Benhida A, et al. Mechanism and kinetics of factor VIII inactivation: study with an IgG4 monoclonal antibody derived from a hemophilia A patient with inhibitor. *Blood* 1998; **92**: 496–506.
18 Spiegel PC Jr., Jacquemin M, Saint-Remy JMR, et al. Structure of a Factor VIII C2 domain:IgG4k Fab complex: Identification of an inhibitory antibody epitope on the surface of Factor VIII. *Blood* 2001; **98**: 13–19.
19 Andersen BR, Terry WD. Gamma G4-globulin antibody causing inhibition of clotting Factor VIII. *Nature* 1968; **217**: 174–5.
20 Satoguina JS, Adjobimey T, Arndts K, et al. Tr1 and naturally occurring regulatory T cells induce IgG4 in B cells through GITR/GITR-L interaction, IL-10 and TGF-beta. *Eur J Immunol* 2008; **38**: 3101–13.
21 Biggs R, Austen DE, Denson KW, et al. The mode of action of antibodies which destroy factor VIII. II. Antibodies which give complex concentration graphs. *Br J Haematol* 1972; **23**: 137–55.
22 Gawryl M, Hoyer L. Inactivation of factor VIII coagulant activity by two different types of human antibodies. *Blood* 1982; **60**: 1103–8.
23 Gilles JG, Lavend'homme R, Peerlinck K, et al. Some factor VIII (FVIII) inhibitors recognise a FVIII epitope(s) that is present only on FVIII–vWF complexes. *Thromb Haemost* 1999; **82**: 40–5.
24 Jacquemin MG, Benhida A, Peerlinck K, et al. A human antibody directed to the factor VIII C1 domain inhibits factor VIII cofactor activity and binding to von Willebrand factor. *Blood* 2000; **95**: 156–65.
25 Peerlinck K, Arnout J, DiGiambattista M, et al. Factor VIII inhibitors in previously treated haemophilia A patients with a double virus-inactivated plasma derived factor VIII concentrate. *Thromb Haemost* 1997; **77**: 80–86.
26 Mariani G, Siragusa S, Kroner BL, et al. Immune tolerance induction in hemophilia A: A review. *Semin Thromb Hemostasis* 2003; **29**: 69–76.
27 Fijnvandraat K, Celie PHN, Turenhout EAM, et al. A human alloantibody interferes with binding of factor IXa to the factor VIII light chain. *Blood* 1998; **91**: 2347–52.
28 Nogami K, Shima M, Nishiya K, et al. Human factor VIII inhibitor alloantibodies with a C2 epitope inhibit factor Xa-catalyzed factor VIII activation: A new anti-factor VIII inhibitory mechanism. *Thromb Haemost* 2002; **87**: 459–65.
29 Nogami K, Shima M, Giddings JC, et al. Circulating factor VIII immune complexes in patients with type 2 acquired hemophilia A and protection from activated protein C-mediated proteolysis. *Blood* 2001; **97**: 669–77.
30 Lacroix-Desmazes S, Horn MP, Bayry J, et al. High prevalence of antibodies with proteolytic activity to Factor VIII in hemophilia A patients with Factor VIII inhibitors. *N Engl J Med* 2002; **346**: 662–7.
31 Dazzi F, Tison T, Vianello F, et al. High incidence of anti-FVIII antibodies against non-coagulant epitopes in haemophilia A patients: a possible role for the half-life of transfused FVIII. *Br J Haematol* 1996; **93**: 688–93.
32 Bray GL, Kroner BL, Arkin S, et al. Loss of high-responder inhibitors in patients with severe hemophilia-A and human immunodeficiency virus type-1 infection—a report from the Multi-Center Hemophilia Cohort Study. *Am J Hematol* 1993; **42**: 375–9.
33 Astermark J, Wang X, Oldenburg J, et al. MIBS Study Group. Polymorphisms in the CTLA-4 gene and inhibitor development in patients with severe hemophilia A. *J Thromb Haemost* 2007; **5**: 263–5.
34 Singer ST, Addiego JE, Reason DC, et al. T lymphocyte proliferative responses induced by recombinant factor VIII in hemophilia A patients with inhibitors. *Thromb Haemost* 1996; **76**: 17–22.
35 Reding MT, Wu H, Krampf M, et al. Sensitization of CD4+ T cells to coagulation factor VIII: response in congenital and acquired hemophilia patients and in healthy subjects. *Thromb Haemost* 2000; **84**: 643–52.
36 Jacquemin MG, Vantomme V, Buhot C, et al. A single mutation Arg2150His regulates the T cell specificity for the factor VIII C1 domain: a molecular mechanism responsible for the higher incidence of inhibitor in mild/moderate hemophilia A patients with mutations in the C1 domain. *Blood* 2003; **101**: 1351–8.
37 James EA, Kwok WW, Ettinger RA, Thompson AR, Pratt KP. T-cell responses over time in a mild hemophilia A inhibitor subject: Epitope identification and transient immunogenicity of the corresponding self-peptide. *J Thromb Haemost* **5**: 2399–407.
38 Dasgupta S, Navarrete AM, Bayry J, et al. A role for exposed mannosylations in presentation of human therapeutic self-proteins to CD4+ T lymphocytes. *Proc Natl Acad Sci USA* 2007; **104**: 8965–70.
39 Dasgupta S, Repessé Y, Bayry J, et al. VWF protects FVIII from endocytosis by dendritic cells and subsequent presentation to immune effectors. *Blood* 2007; **109**: 610–12.

8 Genetic and environmental risk factors for inhibitor development

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Introduction

Hemophilia A and B are caused by an inherited deficiency in factor VIII (FVIII) and IX (FIX), respectively, requiring replacement therapy with the deficient factor. Today, several different concentrates are available, all efficiently restoring the hemostatic defect. Despite all the successes in clinical management over recent years, however, the risk of developing neutralizing FVIII and FIX antibodies still remains [1]. The formation of these antibodies is known to be a T-helper (T_H)-cell-dependent event that involves antigen-presenting cells and B lymphocytes, but the reason why only a fraction of the patients experience this side-effect is not known. For decades, distinction between self and nonself has been considered to be the primary goal of the immune system. In the 1990s, however, Matzinger postulated that the driving force of the immune system is the need to detect and protect against danger, rather than nonself [2]. This concept has gained increasing interest with respect to how it might apply to hemophilia, and today the so-called "danger theory" seems to at least partially explain why some patients are more likely to develop antibodies than others. According to this theory, the deficient factor should not induce a productive immune response after infusion unless it is given in association with additional stimuli of the immune system, i.e., danger signals [3,4]. This chapter will summarize the data available in unrelated and related subjects regarding genetic and environmental factors and discuss how these factors might interact in the process of inhibitor development.

Genetic factors

Data from related and unrelated subjects with hemophilia A clearly indicate that the immunologic outcome of replacement therapy and the risk of developing neutralizing antibodies is to a large extent determined by patient-related genetic factors [5,6]. For example, the immune response to FVIII is similar in up to 80% of family members, significantly higher than expected compared with data from unrelated subjects [6]. Ethnic differences in inhibitor incidence further support the importance of genetic factors. In a meta-analysis of patients with severe hemophilia A, the inhibitor incidence was twice as high in African-Americans compared with Caucasians [7]. Genetic factors that may influence the outcome of replacement therapy include the type of causative gene mutation, the major histocompatibility complex (MHC), and various polymorphic genes coding for immune-regulatory molecules.

Type of causative mutation

The most extensively studied genetic risk factor for inhibitors is the type of causative FVIII mutation. This topic is covered in detail in another chapter and will be only briefly addressed here. Null mutations, i.e., mainly large gene deletions, nonsense mutations and intrachromosomal aberrations, result in the absence of endogenous protein. Patients with these types of mutations are usually at higher risk for the development of inhibitors than patients with missense mutations, small deletions/insertions, and splice site mutations [8,9]. A relatively high risk may also be encountered in patients with splicing errors and mutations causing frame-shifts [10]. The overall lower incidence of inhibitors in patients with hemophilia B (usually <5%) has been attributed to the relatively high frequency of low-risk mutations [11]. However, inhibitors do develop in patients with low-risk mutations, and all patients with high-risk mutations do not develop inhibitors. In addition, family members with the same type of mutation show a high rate of concordance, indicating that the causative mutation will certainly provide the fundament for inhibitor development, but that additional genetic factors will determine the final outcome [12].

Major histocompatibility complex

The MHC on the antigen-presenting cells plays a central role in the immune system and includes the human leukocyte antigen (HLA) class I and II alleles. The class II alleles will determine which FVIII and FIX peptides are presented to the T-cell receptor, and without MHC molecules there will be no immune response elicited. To date, no unequivocal

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associations with inhibitor development have been identified. The HLA class I alleles A3, B7, and C7, as well as the class II alleles DQA0102, DQB0602, and DR15, have all been associated with a slightly higher risk for inhibitor development in unrelated patients, whereas the HLA C2, DQA0103, DQB0603, and DR13 alleles might be protective [13,14]. The reported associations have, however, been weak and not consistent. In the Malmö International Brother Study (MIBS), these alleles were equally distributed between patients with and without inhibitors, and no significant associations with the class II molecules were identified [15]. The weak associations between the MHC molecules and inhibitor formation may be a result of the promiscuous nature of the class II alleles and/or the haplotype inheritance that masks important alleles [16].

Immune-regulatory molecules

Polymorphic genes coding for immune-regulatory factors have been shown to confer susceptibility to antibody-mediated autoimmune diseases, and data on inheritance and ethnic discrepancies in inhibitor risk suggest that these factors will also be important for the immune response to FVIII and FIX in patients with hemophilia. Only a few of the polymorphic candidate genes have been evaluated and, because inhibitor development seems to be a polygenic complex process, it will be important to evaluate all potential candidates to fully appreciate the influence of both genetic and nongenetic determinants. In the MIBS, polymorphic sites in the genes coding for interleukin 10 (IL-10), tumor necrosis factor (TNF)-α and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) were all associated with the risk of experiencing inhibitors [15,17,18].

IL-10 is an important anti-inflammatory cytokine exerting a broad spectrum of activities. This cytokine also enhances the *in vitro* production of all types of immunoglobulins by peripheral blood mononuclear cells in patients with autoimmune diseases [19,20]. In MIBS, a 134 bp-long variant of a CA microsatellite in the promoter region (IL-10.G) was identified in 26.8% of patients with hemophilia A. Thirty-two of these patients (72.7%) developed inhibitors compared with 37.5% of those without the allele [17]. Among all patients with a history of inhibitors, the allele 134 was found in 41.6% compared with 13.8% in inhibitor-negative patients [odds ratio (OR) 4.4; 95% confidence interval (CI) 2.1–9.5; $P < 0.001$]. The association was consistent in a subgroup analysis of patients with severe hemophilia A, and in those with inversion mutations.

TNF-α is an important mediator of inflammatory responses, and several polymorphic sites in the gene have been described. The most extensively studied polymorphism with suggested pathophysiologic effects is a bi-allelic polymorphism at position –308 in the promoter region consisting of a substitution of an A (allele 2) instead of a G (allele 1) [21,22]. Homozygocity for this allele 2 was found in 22 individuals (13.4%), of whom 72.7% had experienced inhibitors (OR 4.0; 95% CI 1.4–11.5; $P = 0.008$) [15]. The association between this genotype and the development of inhibitors was observed in subgroup analyses of those with severe hemophilia A and in those with inversion mutations, and was independent of the IL-10.G microsatellite.

CTLA-4 is a receptor mainly displayed on activated T cells. It mediates a downregulation of the T-cell activity by competing with CD28 for the binding of the B7 molecules, and blockade of this interaction by CTLA-4 antibodies enhances T-cell proliferation and B-cell activity. Several polymorphisms in the CTLA-4 gene have been described, including two single nucleotide polymorphisms (SNPs) [23]. One of these SNPs (C/T) is located in the promoter region of the gene at position –318, of which the T-allele has been associated with an upregulation of the CTLA-4 activity on the activated T cells. Thirty-two patients (25.8%) in the MIBS cohort were T-allele carriers, of whom 31.2% had a history of inhibitor compared with 57.6% of those without the allele T (OR 0.3; 95% CI 0.1–0.8; $P = 0.012$). This suggests that the T-allele might be protective against inhibitor development [18]. None of the patients who were homozygous for the T-allele had a history of inhibitors.

Environmental factors

The impact of environmental risk factors on inhibitor development is not clear, but reports of patients considered low risk for inhibitors who experience them in association with immune system challenges suggest that these factors are potentially of importance. In addition, monozygotic twins discordant for inhibitor status have been described. This would not occur if inhibitor development was a purely genetically determined event [6]. Factors attracting the greatest interest are age at start of treatment, the use of prophylaxis, the type of factor concentrate used, mode of administration, as well as treatment, and the intensity of treatment, in association with immune system challenges.

Age at start of treatment

An inverse relationship between age at start of treatment and the risk of developing antibodies against FVIII has been suggested in studies focusing on age at start without considering other potentially confounding factors [24,25]. An immunologic explanation for increased risk accompanying treatment at an early age is that the immature immune system is more susceptible than the mature system. These findings have not been confirmed, however, in later studies that also considered genetic factors [26–28]. Thus, age may not be the major determinant. Instead, the reasons for treatment together with the genetic risk profile of the patient are likely to carry greater importance.

Prophylaxis

The hypothesis of increasing risk of inhibitor development when administering replacement therapy in the presence of a danger signal has raised the question of whether prophylactic treatment at a young age would confer a protective effect. Interestingly, the dosing in prophylaxis will, in many cases, be similar to that of low-dose immune tolerance therapy, and the use of prophylaxis has indeed been associated with a lower frequency of inhibitors in recent studies, even after adjustment for confounding factors [26,28,29]. In countries such as Sweden, however, in which primary prophylaxis has been extensively used for several decades, the inhibitor incidence seems to be similar to that of other cohorts treated on demand [30]. Thus, it is not possible to state that prophylaxis per se will prevent inhibitor development, but further studies evaluating prophylaxis as the true first reason for treatment, and in the absence of immune system challenges, should be performed. As prophylaxis is the state of the art, there should be no major obstacles to performing these studies, which, in turn, will also shed light on the optimum time for starting treatment in young children.

Type of factor concentrate

The availability of safe and effective recombinant replacement therapy has been a major achievement during the last decades. However, concerns have been raised that high-purity recombinant products might be more antigenic than plasma-derived concentrates with or without the von Willebrand factor (VWF) and associated with an increased risk of inhibitor development [27,31,32]. These concerns are based primarily on findings of retrospective studies of small and not comparable cohorts, unadjusted for confounding factors. It is also worth pointing out that the product-related effects appear inconsistent when only high-titer (i.e., more clinically relevant) inhibitors are evaluated [32]. Direct comparisons of risk for inhibitor development between plasma-derived and recombinant products do not exist; and in some studies no significant differences between the two types of products have been reported [33,34]. Furthermore, the reported incidence of inhibitors in cohorts of previously untreated patients treated with recombinant products is similar to that observed in cohorts treated primarily with plasma-derived concentrates [35]. Switching from plasma-derived to recombinant products in previously treated patients seems to carry only a small risk of inhibitor development, also indicating that the product antigenicity may not be very different [34]. The possibility that plasma-derived products may be associated with an immunosuppressive effect owing to immunomodulating contaminants and/or the presence of the VWF cannot be completely ruled out [36–38], and there are studies under development to address product-related antigenicity. To date, however, there is no evidence to support the use of plasma-derived products in favor of recombinant agents in order to reduce inhibitor risk in young patients.

Mode of administration

Administration of the deficient factor has traditionally been performed by bolus injections. However, in order to avoid deep troughs and unnecessarily high levels of FVIII, as well as to potentially reduce the amount of factor required to reach a particular hemostatic level, the use of continuous infusion has recently emerged as an alternative treatment modality. Reports of *de novo* inhibitors in patients considered to be at low risk have raised the question of whether the way the infused factor is presented to the immune system has an impact on risk [39,40]. This issue is of particular interest and concern for patients with milder forms of the disease. In most studies, however, both continuous infusion and bolus injections have been used, and in some large centers significant numbers of patients have been treated with continuous infusion with no indication of an increased incidence of inhibitors. Therefore, at least in patients with severe disease, there appears to be no evidence on which to base a recommendation that continuous infusion should be avoided.

Immune system challenges

Conditions associated with an inflammatory response will, from a theoretical point of view, have the potential to provide danger signals, modulate the immune response, and increase the risk of inhibitor formation during replacement therapy. These conditions include tissue damage with extravascular exposure to the deficient factor, severe infections, and immunization. Therefore, if possible, it may be advisable to avoid treatment in association with these types of events, in particular at the start of treatment in young children [41]. There is, however, a lack of studies in the literature that address this issue. In a cohort study conducted in Italy, challenge to the immune system at start of treatment was not associated with inhibitor development [29]. On the other hand, treatment in association with severe tissue damage, and the need for intensive treatment for several days, has been linked to a higher frequency of inhibitors [28,42]. Prospective studies of genetically well-characterized cohorts are needed to better appreciate the reasons for first infusions and the impact of immune system challenges.

Conclusion

The immunologic outcome of replacement therapy and the formation of antibodies to FVIII and FIX seem largely to be genetically determined. However, in accordance with the danger theory, environmental factors will certainly have the potential to modulate the immune response in many patients, and the genetic and environmental factors will likely intervene at the immune regulatory level. The cell repertoire, which to some extent will be determined by the causative mutation, and the MHC will provide the platform for the antibodies to

develop. The individual genetic profile of immune-regulatory molecules will then be additive and create a "safe" or "unsafe" platform for inhibitor formation. In patients with a "safe" platform, i.e., those with a causative factor mutation with the ability to delete T-cell clones recognizing dominant immunogenic epitopes, HLA class II alleles binding only nonimmunogenic peptides, and favorable profiles regarding the polymorphisms in immune-regulatory molecules, the risk of inhibitor development will be low, even in the presence of danger signals. On the other hand, in patients with an "unsafe" platform, the threshold for inhibitor development might be reached by the genetic profile itself when the deficient factor is introduced, or reached when the immune system becomes challenged by danger signals. This schematic model remains to be validated, but it might provide an understanding of the intricacy of the immune response. Modifying pathways and T-regulatory cells will further add to the complexity of the system. A better understanding of the risk factors for inhibitor development may, in the future, allow clinicians to calculate a predictive risk score for each patient, a score that could guide management with the goal of minimizing inhibitor risk.

References

1 Astermark J. Why do inhibitors develop? Principles of and factors influencing the risk for inhibitor development in haemophilia. *Haemophilia* 2006; **12**(Suppl. 3): 52–60.

2 Matzinger P. Tolerance, danger, and the extended family. *Ann Rev Immunol* 1994; **12**: 991–1045.

3 Pfistershammer K, Stöckl J, Siekmann J, Turecek PL, Schwarz HP, Reipert BM. Recombinant factor VIII and factor VIII–von Willebrand factor complex do not present danger signals for human dendritic cells. *Thromb Haemost* 2006; **96**: 309–16.

4 Reipert BM, van Helden PMW, Schwartz H-P, Hausl C. Mechanism of action of immune tolerance induction against factor VIII in patients with congenital haemophilia A and factor VIII inhibitors. *Br J Haematol* 2006; **136**: 12–25.

5 Gill JC. The role of genetics in inhibitor formation. *Thromb Haemost* 1999; **82**: 500–4.

6 Astermark J, Berntorp E, White GC, Kroner BL, and the MIBS Study Group. The Malmö International Brother Study (MIBS): Further support for genetic predisposition to inhibitor development in hemophilia patients. *Haemophilia* 2001; **7**: 267–72.

7 Scharrer I, Bray GL, Neutzling O. Incidence of inhibitors in haemophilia A patients—A review of recent studies of recombinant and plasma-derived factor VIII concentrates. *Haemophilia* 1999; **5**: 145–54.

8 Schwaab R, Brackman HH, Meyer C, *et al.* Haemophilia A: Mutation type determines risk of inhibitor formation. *Thromb Haemost* 1995; **74**: 1402–6.

9 Oldenburg J, El-Maarri O, Schwaab R. Inhibitor development in correlation to factor VIII genotypes. *Haemophilia* 2002; **8**(Suppl. 2): 23–9.

10 Boekhorst J, Lari GR, D'oiron R, *et al.* Factor VIII genotype and inhibitor development in patients with haemophilia A: Highest risk in patients with splice site mutation. *Haemophilia* 2008; **14**: 729–35.

11 Ljung RC. Gene mutations and inhibitor formation in patients with hemophilia B. *Acta Haematol* 1995; **94**(Suppl. 1): 49–52.

12 Astermark J, Oldenburg J, Escobar M, White GC 2nd, Berntorp E; Malmö International Brother study group. The Malmö International Brother Study (MIBS). Genetic defects and inhibitor development in siblings with severe hemophilia A. *Haematologica* 2005; **90**: 924–31.

13 Oldenburg J, Picard JK, Schwaab R, Brackmann HH, Tuddenham EG, Simpson E. HLA genotype of patients with severe haemophilia A due to intron 22 inversion with and without inhibitors of factor VIII. *Thromb Haemost* 1997; **77**: 238–42.

14 Hay CR, Ollier W, Pepper L, Cumming A, Keeney S, Goodeve AC, Colvin BT, Hill FG, Preston FE, Peake IR. HLA class II profile: A weak determinant of factor VIII inhibitor development in severe haemophilia A. UKHCDO Inhibitor Working Party. *Thromb Haemost* 1997; **77**: 234–7.

15 Astermark J, Oldenburg J, Carlson J, Pavlova A, Kavakli K, Berntorp E, Lefvert AK. Polymorphisms in the *TNFA* gene and the risk of inhibitor development in patients with hemophilia A. *Blood* 2006; **108**: 3739–45.

16 White GC 2nd, Kempton CL, Grimsley A, Nielsen B, Roberts HR. Cellular immune responses in hemophilia: Why do inhibitors develop in some, but not all hemophiliacs? *J Thromb Haemost* 2005; **3**: 1676–81.

17 Astermark J, Oldenburg J, Pavlova A, Berntorp E, Lefvert AK; MIBS Study Group. Polymorphisms in the IL10 but not in the *IL1beta* and *IL4* genes are associated with inhibitor development in patients with hemophilia A. *Blood* 2006; **107**: 3167–72.

18 Astermark J, Wang X, Oldenburg J, Berntorp E, Lefvert AK; MIBS Study Group. Polymorphisms in the *CTLA-4* gene and inhibitor development in patients with severe hemophilia A. *J Thromb Haemost* 2007; **5**: 263–5.

19 Llorente L, Zou W, Levy Y, Richaud-Patin Y, Wijdenes J, Alcocer-Varela J, Morel-Fourrier B, Brouet JC, Alarcon-Segovia D, Galanaud P, Emilie D. Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J Exp Med* 1995; **181**: 839–44.

20 Zhou Y, Giscombe R, Huang D, Lefvert AK. Novel genetic association of Wegener's granulomatosis to the interleukin-10 gene. *J Rheumatol* 2002; **29**: 317–20.

21 Wilson AG, di Giovine FS, Blakemore AI, Duff GW. Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum Mol Genet* 1992; **1**: 353.

22 Huang DR, Pirskanen R, Matell G, Lefvert AK. Tumour necrosis factor-alpha polymorphism and secretion in myasthenia gravis. *J Neuroimmunol* 1999; **94**: 165–71.

23 Giscombe R, Wang X, Huang D, Lefvert AK. Coding sequence 1 and promoter single nucleotide polymorphisms in the *CTLA-4* gene in Wegener's granulomatosis. *J Rheumatol* 2002; **29**: 950–3.

24 Lorenzo JI, Lopez A, Altisent C, Aznar JA. Incidence of factor VIII inhibitors in severe haemophilia: The importance of patient age. *Br J Haematol* 2001; **113**: 600–3.

25 van der Bom JG, Mauser-Bunschoten EP, Fischer K, van den Berg HM. Age at first treatment and immune tolerance to factor VIII in severe hemophilia. *Thromb Haemost* 2003; **89**: 475–9.

26 Santagostino E, Mancuso ME, Rocino A, *et al.* Environmental risk factors for inhibitor development in children with haemophilia A: A case–control study. *Br J Haematol* 2005; **130**: 422–7.

27 Chalmers EA, Brown SA, Keeling D, *et al.* Early factor VIII exposure and subsequent inhibitor development in children with severe haemophilia A. *Haemophilia* 2007; **13**: 149–55.

28 Gouw SC, van der Bom JG, van den Berg HM, for the CANAL study group. Treatment-related risk factors of inhibitor development in previously untreated patients with haemophilia A; The CANAL cohort study. *Blood* 2007; **109**: 4648–54.
29 Morado M, Villar A, Jiménez Yuste V, Quintana M, Hernandez Navarro F. Prophylactic treatment effects on inhibitor risk: Experience in one centre. *Haemophilia* 2005; **11**: 79–83.
30 Knobe KE, Sjörin E, Tengborn LI, Petrini P, Ljung RCR. Inhibitors in the Swedish population with severe haemophilia A and B: A 20-year survey. *Acta Paediatr* 2002; **91**: 910–14.
31 Wight J, Paisley S. The epidemiology of inhibitors in haemophilia A: A systematic review. *Haemophilia* 2003; **9**: 418–35.
32 Goudemand J, Rothschild C, Demiguel V, *et al.*; FVIII-LFB and Recombinant FVIII study groups. Influence of the type of factor VIII concentrate on the incidence of factor VIII inhibitors in previously untreated patients with severe hemophilia A. *Blood* 2006; **107**: 46–51.
33 Kreuz W, Ettinghousen CE, Auerswald G, *et al.* Epidemiology of inhibitors and current treatment strategies. *Haematologica* 2003; **88**(Suppl. 9): 17–20.
34 Gouw SC, van der Bom JG, Auerswald G, *et al.* Recombinant versus plasma-derived factor VIII products and the development of inhibitors in previously untreated patients with severe hemophilia A: The CANAL cohort study. *Blood* 2007; **109**: 4693–7.
35 Kreuz W, Gill JC, Rothschild C, *et al.* Full-length sucrose-formulated recombinant factor VIII for treatment of previously untreated or minimally treated young children with severe haemophilia A. *Thromb Haemost* 2005; **93**: 457–67.
36 Wadhwa M, Dilger P, Tubbs J, Mire-Sluis A, Barrowcliffe T, Thorpe R. Identification of transforming growth factor-beta as a contaminant in factor VIII concentrates: A possible link with immunosuppressive effects in hemophiliacs. *Blood* 1994; **84**: 2021–30.
37 Astermark J, Ekman M, Berntorp E. No effect of a new second-generation B-domain-deleted recombinant product on lymphocyte transformation *in vitro*: A study of plasma-derived and recombinant products. *Br J Haematol* 1997; **99**: 289–94.
38 Dasgupta S, Repesse Y, Bayry J, *et al.* VWF protects FVIII from endocytosis by dendritic cells and subsequent presentation to immune effectors. *Blood* 2007; **109**: 610–12.
39 Sharathkumar A, Lillicrap D, Blanchette VS, *et al.* Intensive exposure to factor VIII is a risk factor for inhibitor development in mild hemophilia A. *J Thromb Haemost* 2003; **1**: 1228–36.
40 von Auer Ch, Oldenburg J, von Depka M, *et al.* Inhibitor development in patients with hemophilia A after continuous infusion of FVIII concentrates. *Ann NY Acad Sci* 2005; **1051**: 498–505.
41 Oldenburg J, Schröder J, Brackmann H-H, Müller-Reible C, Schwaab R, Tuddenham E. Environmental and genetic factors influencing inhibitor development. *Sem Hematol* 2004; **41**(Suppl. 1): 82–8
42 Gouw SC, Van Den Berg HM, Le Cessie S, van der Bom JG. Treatment characteristics and the risk of inhibitor development: A multicentre cohort study among previously untreated patients with severe hemophilia A. *J Thromb Haemost* 2007; **5**: 1383–90.

9 Inhibitors to factor VIII: mild and moderate hemophilia

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Introduction

Until the late 1990s, inhibitors in mild/moderate hemophilia A were considered to be very rare. However, since the publication of Hay *et al.* [1] in 1998 on behalf of the UK Haemophilia Centre Doctors' Organisation (UKHCDO), it has been appreciated that inhibitors in mild/moderate haemophilia are more frequent than previously thought. Clinical problems associated with inhibitors in mild/moderate hemophilia are often considerable, since in the majority of cases adult patients are confronted with a change in phenotype from mild/moderate to severe and they suddenly experience spontaneous severe bleeding. Although some of the risk factors for inhibitor development are similar to those in severe hemophilia, others are specific for mild/moderate hemophilia. The study of the immune response in mild/moderate hemophilia A can help to elucidate some of the mechanisms underlying inhibitor formation and disruption of tolerance. Treatment of bleeding episodes and eradication of inhibitors in mild/moderate hemophilia require specific management, and special attention should go to the prevention of this complication.

Incidence and prevalence

Patients with mild/moderate hemophilia are at lower risk of inhibitor development than severely affected patients. The prevalence of these inhibitors has been estimated to be between 3% and 13% [2–4]. In a prospective study of inhibitor incidence among 1306 hemophilia A patients, only 6% of the inhibitors were found in patients with factor VIII >0.03 IU/mL [5]. Sixteen (28%) of 57 new inhibitors reported between January 1990 and January 1997 in the UKHCDO inhibitor register arose in patients with mild or moderate hemophilia [1]. The annual incidence of inhibitors in the UK was 3.5 per 1000 registered with severe hemophilia and 0.84 per 1000 patients registered with mild/moderate hemophilia [6].

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Clinical presentation

Usually the presence of an inhibitor in patients with mild/moderate hemophilia is suggested by a change in bleeding pattern: patients suddenly start to experience severe spontaneous bleeding, where previously they used to bleed only after trauma or surgery. This change in bleeding pattern is explained by cross-reactivity of the inhibitor with the mutated factor VIII of the patient resulting in a residual factor VIII:c level of <0.01 IU/mL [7–9]. The bleedings occur often in muscles and joints, as in severe congenital hemophilia, but sometimes the bleeding pattern is more reminiscent of acquired hemophilia with the occurrence of large cutaneous bruising, and gastrointestinal and urogenital bleeding [1]. Occasionally, there is no change in residual factor VIII level but an inhibitor is detected in the Bethesda assay and/or there is lack of efficiency of factor VIII transfusions [9–11]. In some cases, the specificity of the immune response reverts over time from neutralization of both mutated self and transfused normal factor VIII to tolerance to self, resulting in a recovery of the original basal factor VIII level and response to desmopressin, despite the persistence of antibodies to exogenous factor VIII [1,7,9,10].

Risk factors

Intensive exposure to factor VIII

Inhibitors in mild/moderate hemophilia occur more commonly later in life, and an episode of intensive treatment with factor VIII concentrate (for bleeding, trauma, or surgery) seems to precede detection of the inhibitor in most reported cases. In the series reported by Hay *et al.* [1], 16 out of 26 inhibitors were detected after such intensive replacement therapy and in this series no particular concentrate was implicated. Intensive exposure to factor VIII as a risk factor for inhibitor development in mild hemophilia A was confirmed in a recent publication from Canada [12]. The overall incidence of inhibitors in their population of boys (aged between 0 and 18 years) with mild hemophilia A (n = 54) was 7.4%. When the analysis was restricted to patients exposed to factor VIII the incidence was 14% (4/29), and patients who received factor VIII as a continuous infusion developed inhibitors in four out of five (57%)

cases. At this moment it is not clear whether the risk is confined to high exposure to factor VIII alone or whether additional risk is associated with the way of administering the concentrate (bolus injection versus continuous infusion) [13]. An answer to this question will likely require a prospective multicenter study.

Genetic background

In severe hemophilia the risk of inhibitor formation is associated with the type of mutation. More disruptive mutations in the factor VIII gene, such as intron 22 inversions, large gene deletions, and stop codons, are associated with a ~35% risk of inhibitor formation, compared with only about 5% in those with missense mutations and small deletions [14]. Missense mutations in the light chain are more often (12%) associated with inhibitors than are missense mutations in other parts of the factor VIII gene (3.9%) [14]. In patients with mild/moderate hemophilia and inhibitors certain missense mutations seem to predispose to inhibitor formation. In the series of Hay *et al.* [1], seven out of nine mutations were clustered in a region at the junction between the C1 and C2 domain. The two remaining mutations affected the A2 domain. Clustering of the mutations in these regions has been confirmed in most other reported cases of mild/moderate hemophilia with inhibitor and some particular mutations such as Arg2150His and Arg593Cys seem to be over-represented [1,7,9,10,15–18]. Further information is still required on the influence of polymorphisms of genes involved in the regulation of the immune response to factor VIII in patients with mild/moderate hemophilia A [19,20].

Analysis of the immune response to factor VIII in mild/moderate hemophilia A

To determine why some mutations located in the A2, C1, or C2 domains of the factor VIII molecule are more frequently associated with the presence of inhibitor, the humoral and cellular responses to factor VIII were analyzed at the polyclonal and clonal level.

Analysis of factor VIII produced by patients with mild/moderate hemophilia A demonstrated that mutations at residues Arg2150, Arg2159, or Ala2201 eliminate factor VIII epitopes (antigenic determinants) recognized by monoclonal inhibitor antibodies [21–24], which confirmed observations made using patients' polyclonal antibodies [7,10,25].

The T-cell response to factor VIII was studied in a mild hemophilia A patient carrying an Arg2150His substitution in the C1 domain and who presented with a high-titer inhibitor toward normal but not self factor VIII. The factor VIII-specific T cells of this patient recognized a peptide encompassing residue Arg2150, the residue mutated in the patient's factor VIII gene, and did not recognize recombinant factor VIII carrying the substitution Arg2150His. Thus, the C1 domain of wild-type factor VIII contains T-cell epitopes that are absent in factor VIII carrying the mutation Arg2150His [26].

These observations demonstrate that Arg2150His factor VIII and normal factor VIII can be distinguished by the immune system not only at the B-cell level, but also at the T-cell level. Recently, similar observations have been made with a patient carrying a mutation A2201P [27], suggesting that this type of phenomenon may occur in patients carrying mutations responsible for mild/moderate hemophilia A and predisposing to inhibitor formation.

Treatment

Bleeding episodes

Bleeding episodes in patients with mild/moderate haemophilia who developed an inhibitor are often particularly severe and sometimes life threatening. Bypass therapy with activated prothrombin complex concentrates or recombinant activated factor VII can be used to control bleeding and has the advantage of avoiding anamnesis. Some patients can be treated successfully with desmopressin, especially those whose basal factor VIII level did not significantly decrease and whose inhibitor does not seem to cross-react with their endogenous factor VIII [1,9,10] or once adequate circulating factor VIII levels have returned. Desmopressin does not cause anamnesis in those patients despite the presence of high-responding inhibitors [1].

Inhibitor eradication

Published data on immune tolerance induction in patients with mild/moderate hemophilia and inhibitors are very scarce. In the series reported by Hay *et al.* [1], immune tolerance induction was attempted in eight patients using different regimens. The Malmö regime [high-dose factor VIII combined with cyclophosphamide and intravenous immunoglobulin G (IgG)] was used successfully in two patients and with a partial response in a further two patients, the Van Creveld regime (low-dose factor VIII every other day) was used unsuccessfully in one patient and with partial success in a further patient, and the Bonn regime was used unsuccessfully in one patient and with partial success in another patient. The overall success rate of immune tolerance of two out of eight patients seems lower than the reported success rate in severe hemophilia.

Other reported treatments have included immunomodulatory drugs such as corticosteroids, cyclophosphamide, anti-CD20 monoclonal antibody rituximab [8,28–31] and avoidance of re-exposure to factor VIII using desmopressin and bypassing agents to treat bleeding episodes [32]. Currently available data are not sufficient to offer evidence-based advice on the optimal treatment of inhibitors in patients with mild/moderate hemophilia A, and the management of these patients

remains controversial at this point. Preliminary data from a retrospective and prospective data collection in France and Belgium [33,34] suggest that immune tolerance induction could be more effective than no specific treatment or immunomodulating drugs in preventing risk of anamnesis of the inhibitor after re-exposure to factor VIII.

Prevention

Maximal use of desmopressin for the treatment of patients with mild/moderate hemophilia A is certainly useful to prevent the development of inhibitors in these patients. Avoidance of intensive courses of treatment with factor VIII concentrates has to be considered, especially in those patients known to harbor one of the high-risk mutations or having a relative who developed an inhibitor. It is not clear at this moment whether administration of factor VIII as bolus injections or as continuous infusion infers different risks. Identification of the underlying mutation in patients with mild/moderate hemophilia A is useful to have an indication of their risk of inhibitor formation.

Conclusion

The occurrence of an inhibitor in a patient with mild/moderate hemophilia A is often a dramatic event. Bleeding episodes can be particularly severe, can force patients to change their lifestyle completely, and may be life threatening. Avoidance of treatment with factor VIII concentrates by using desmopressin where possible is the single most effective way to prevent this complication in mild/moderate hemophilia A patients. It is not clear at this moment whether immune tolerance induction, immune suppression, or a combination of both should be used to eradicate the inhibitor. Again, avoidance of exposure to factor VIII, by using bypassing agents (FEIBA or rFVIIa) and desmopressin to treat bleeding episodes, might be an interesting option.

References

1 Hay CR, Ludlam CA, Colvin BT, *et al.* Factor VIII inhibitors in mild and moderate-severity haemophilia A. *Thromb Haemost* 1998; **79**: 762–6.

2 Lusher JM, Arkin S, Abildgaard CF, *et al.* Recombinant factor VIII for the treatment of previously untreated patients with haemophilia A. *N Engl J Med* 1993; **328**: 453–9.

3 Sultan Y, and the French Haemophilia Study Group. Prevalence of inhibitors in a population of 3435 haemophilia patients in France. *Thromb Haemost* 1992; **67**: 600–2.

4 Rizza CR, Spooner RGD. Treatment of haemophilia and related disorders in Britain and Northern Ireleand during 1976–80: report on behalf of the directors of haemophilia centres in the United Kingdom. *Br Med J* 1983; **286**: 929–32.

5 McMillan CW, Shapiro SS, Whitehurst D, *et al.* The natural history of factor VIII:c inhibitors in patients with haemophilia A: A national cooperative study. II; Observations on the initial development of factor VIII:c inhibitors. *Blood* 1988; **71**: 344–8.

6 Rizza CR, Spooner RJD, Giangrande PLF, on behalf of the UK Haemophilia Centre Doctors' Organisation (UKHCDO). Treatment of haemophilia in the UK 1981–96. *Haemophilia* 2001; **7**: 349–59.

7 Fijnvandraat K, Turenhout EAM, van den Brink EN, *et al.* The missense mutation Arg593 → Cys is related to antibody formation in a patient with mild haemophilia A. *Blood* 1997; **89**: 4371–7.

8 Vlot AJ, Wittebol S, Strengers PFW, *et al.* Factor VIII inhibitor in a patient with mild haemophilia A and an Asn618-Ser mutation responsive to immune tolerance induction and cyclophosphamide. *Br J Haematol* 2002; **117**: 136–40.

9 Santagostino E, Gringeri A, Tagliavacca L, *et al.* Inhibitors to factor VIII in a family with mild haemophilia: Molecular characterization and response to factor VIII and desmopressin. *Thromb Haemost* 1995; **74**: 619–21.

10 Peerlinck K, Jacquemin M, Arnout J, *et al.* Antifactor VIII antibody inhibiting allogeneic but not autologous factor VIII in patients with mild haemophilia A. *Blood* 1999; **93**: 2267–73.

11 Kesteven PJ, Holland LJ, Lawrie AS, *et al.* Inhibitor to Factor VIII in mild haemophilia. *Thromb Haemost* 1984; **52**: 50–52.

12 Sharathkumar A, Lillicrap D, Blanchette VS, *et al.* Intensive exposure to factor VIII is a risk factor for inhibitor formation in mild haemophilia A. *J Thromb Haemost* 2003; **1**: 1228–36.

13 von Auer Ch, Oldenburg J, von Depka M, *et al.* Inhibitor development in patients with hemophilia A after continuous infusion of FVIII concentrates. *Ann N Y Acad Sci* 2005; **1051**: 498–505.

14 Goodeve AC, Peake IR. The molecular basis of haemophilia A: Genotype–phenotype relationships and inhibitor development. *Semin Thromb Haemost* 2003; **29**: 23–30.

15 Thompson AR, Murphy MEP, Liu M, *et al.* Loss of tolerance to exogenous and endogenous factor VIII in a mild haemophilia A patient with an Arg593 to Cys mutation. *Blood* 1997; **90**: 1902–10.

16 Fernandez-Lopez O, Garcia-Lozano JR, Nunez-Vasquez R, *et al.* The spectrum of mutations in Southern Spanish patients with hemophilia A and identification of 28 novel mutations. *Haematologica* 2005; **90**: 707–10.

17 Knobe KE, Villoutreix BO, Tengborn LI, *et al.* Factor VIII inhibitors in two families with mild haemophilia A: Structural analysis of the mutations. *Haemostasis* 2000; **30**: 268–79.

18 Franchini M, Girelli D, Olivieri O, *et al.* Tyr2105Cys mutation in exon 22 of FVIII gene is a risk factor for the development of inhibitors in patients with mild/moderate haemophilia A. *Haemophilia* 2006; **12**: 448–51.

19 Astermark J, Wang X, Oldenburg J, *et al.* Polymorphisms in the *CTLA-4* gene and inhibitor development in patients with severe hemophilia A. *J Thromb Haemost* 2007; **5**: 263–5.

20 Astermark J, Oldenburg J, Carlson, *et al.* Polymorphisms in the *TNFA* gene and the risk of inhibitor development in patients with hemophilia A. *Blood* 2006; **108**: 3739–45.

21 Jacquemin M, Benhida A, Peerlinck K, *et al.* A human antibody directed to the factor VIII C1 domain inhibits factor VIII cofactor activity and binding to von Willebrand factor. *Blood* 2000; **95**: 156–63.

22 Suzuki H, Shima M, Arai M, *et al.* Factor VIII Ise (R2159C) in a patient with mild haemophilia A, an abnormal Factor VIII with retention of function but modification of C2 epitopes. *Thromb Haemost* 1997; **77**: 862–7.

23 d'Oiron R, Lavergne JM, Lavend'homme R, *et al.* Deletion of Alanine 2201 in the FVIII C2 domain results in mild haemophilia A by impairing FVIII binding to vWF and phospholipids and

destroys a major FVIII antigenic determinant involved in inhibitor development. *Blood* 2004; **103**: 155–7.
24 Jacquemin M, Benhida A, Peerlinck K, *et al.* A human antibody directed to the factor VIII C1 domain inhibits factor VIII cofactor activity and binding to von Willebrand factor. *Blood* 2000; **95**: 156–63.
25 Gilles JG, Lavend'homme R, Peerlinck K, *et al.* Some factor VIII (FVIII) inhibitors recognise a FVIII epitope(s) that is present only on FVIII–vWF complexes. *Thromb Haemost* 1999; **82**: 40–45.
26 Jacquemin M, Vantomme V, Buhot C, *et al.* CD4+ T-cell clones specific for wild-type factor VIII: A molecular mechanism responsible for a higher incidence of inhibitor formation in mild/moderate haemophilia A. *Blood* 2003; **101**: 1351–8.
27 James EA, Kwok WW, Ettinger RA, *et al.* T-cell responses over time in a mild hemophilia A inhibitor subject: Epitope identification and transient immunogenicity of the corresponding self-peptide. *J Thromb Haemost* 2007; **5**: 2399–407.
28 Capel P, Toppet M, Van Remoor E, *et al.* Factor VIII inhibitor in mild haemophilia. *Br J Haemat* 1986; **62**: 786–7.
29 White B, Cotter M, Byrne M, *et al.* High responding factor VIII inhibitors in mild haemophilia-is there a link with recent changes in clinical practice? *Haemophilia* 2000; **6**: 113–15.
30 Carcao M, St Louis J, Poon MC, *et al.* Inhibitor Subcommittee of Association of Hemophilia Clinic Directors of Canada. Rituximab for congenital haemophiliacs with inhibitors: A Canadian experience. *Haemophilia* 2006; **12**: 7–18.
31 Dunkley S, Kershaw G, Young G, *et al.* Rituximab treatment of mild haemophilia A with inhibitors: A proposed treatment protocol. *Haemophilia* 2006; **12**: 663–7.
32 Robbins D, Kulkarni R, Gera R, *et al.* Successful treatment of high titer inhibitors in mild haemophilia A with avoidance of factor VIII and immunosuppressive therapy. *Am J Hematol* 2001; **68**: 184–8.
33 d'Oiron R, Volot F, Reynaud J, *et al.* Impact of choice of treatment for bleeding episodes on inhibitor outcome in patients with mild/moderate hemophilia A and inhibitors. *Semin Hematol* 2006; **43**(Suppl. 1): S3–9.
34 d'Oiron R, Pipe SW, Jacquemin M. Mild/moderate haemophilia A: New insights into molecular mechanisms and inhibitor development. *Haemophilia* 2008; **14**(Suppl. 3): 138–46.

10 Inhibitors to factor VIII/IX: immune tolerance

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Introduction

Given the inhibitor-associated morbidity resulting from limited treatment options, antibody eradication is the ultimate goal of inhibitor management. The only proven strategy for achieving antigen-specific tolerance to factor VIII (FVIII) or factor IX (FIX) is immune tolerance induction (ITI) therapy. Although first reported almost 30 years ago [1], our current knowledge about ITI still derives from several small cohort studies and three larger ITI registries, as recently summarized [2]. Consequently, practitioners and patients encounter more questions than answers when considering ITI for inhibitor eradication.

This chapter discusses our current knowledge of host and treatment factors, as well as supportive care initiatives known, or suspected to influence, ITI outcome in patients with FVIII or FIX antibodies. In the eradication of FVIII inhibitors, questions concerning the choice of therapeutic product and/or dosing regimen generate the most controversy. In the case of severe hemophilia B, the most significant question revolves around the safest and most effective strategy to eradicate FIX antibodies associated with allergic manifestations. The ongoing clinical trials designed to clarify several of these polarizing issues will be reviewed. Furthermore, the current practice of ITI for low-titer/responding inhibitors and inhibitors developing in moderate/mild hemophilia A patients will also be discussed. Finally, this chapter will both summarize the currently recommended practice and explore possible future approaches to ITI, including immune-modulatory strategies. A case will be made to move beyond empiric therapeutics and toward a risk-stratified and evidence-based approach to inhibitor eradication.

Defining immune tolerance induction outcome

Historically, ITI success rates have been defined by variable clinical and laboratory endpoints. By international consensus, successful ITI in hemophilia A is currently defined as both an undetectable inhibitor titer [≤0.6 Bethesda units (BU) by Bethesda or Nijmegen assays], and normalized FVIII pharmacokinetics. Normalized FVIII pharmacokinetics is further defined as a plasma FVIII recovery ≥66% of expected and a half-life ≥6 h, determined following a 72-h FVIII exposure-free period [*Consensus Proceedings from the Second International Conference on Immune Tolerance Therapy.* Bonn, Germany, 1997 (unpublished)]. Once successful ITI is achieved, usually within the first 33 months of therapy [3], long-term FVIII prophylaxis is commonly instituted. However, the contribution of prophylaxis to the published ITI maintenance rate of 93% remains unclear [4]. The International ITI (I-ITI) Study has also defined ITI partial success by both a reduction in inhibitor titer to ≤5 BU without normalization of FVIII pharmacokinetics, and absent treatment—limiting anamnesis over a prolonged observation period [5].

Drawing from international consensus criteria and the analysis of the International Immune Tolerance Registry (IITR), the I-ITI study has defined ITI failure by either two criteria: (i) Failure to attain the definition of success within 33 months of uninterrupted ITI; or (ii) failure to demonstrate a progressive 20% reduction in inhibitor titer over each successive 6-month period of uninterrupted ITI, beginning 3 months after initiation to allow for expected anamnesis [3,5]. This definition implies a minimum ITI trial period of 9 months before failure is declared. The European Haemophilia Standardisation Board (EHSB) adopted these definitions with minor modifications [6]. The International Consensus Panel (ICP) suggested that ITI outcome would be more scientifically defined by immunologic response [7]. To that end, preliminary data suggest that quantitative shifts in IgG1 and IgG4 subclasses during ITI may predict outcome [8].

A specific consensus definition of ITI success in hemophilia B has not yet been developed. In practice, therapeutic endpoints are extrapolated from those derived for ITI in hemophilia A.

Immune tolerance: factor VIII inhibitors

The role of host factors in immune tolerance induction outcome

The host factors implicated in ITI outcome are summarized in Figure 10.1. The most significant of these implicate the patient's own immune response to FVIII. The IITR, German

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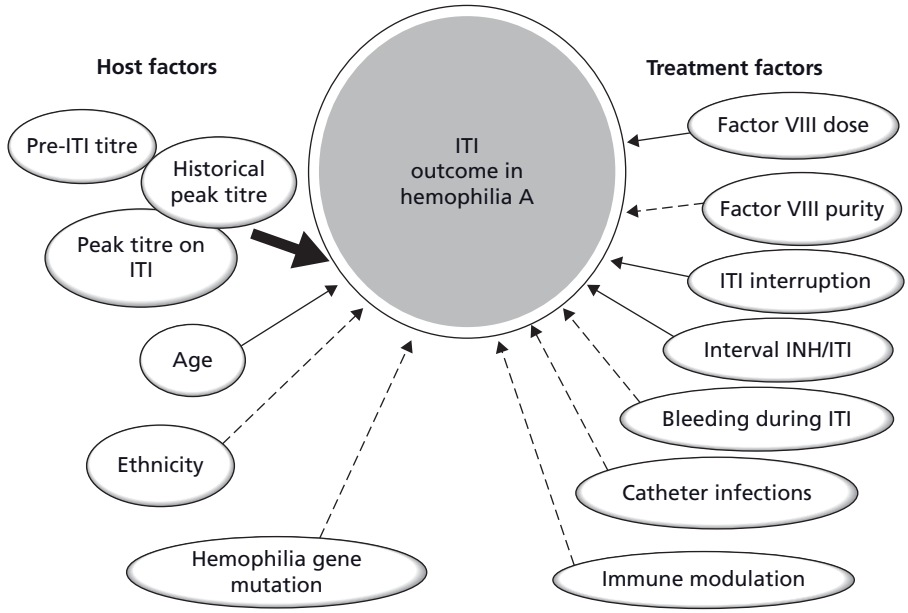

Figure 10.1 Host- and treatment-related factors known or suspected of influencing immune tolerance outcome in severe hemophilia A. The thickness of the arrows reflects level of evidence in published literature supporting each potential risk-modifying factor. INH, inhibitor; ITI, immune tolerance induction.

Table 10.1 Preinduction titer as an outcome predictor of immune tolerance induction (ITI) success in severe high-responder (HR) hemophilia A subjects in the North American Immune Tolerance Registry (NAITR) [10].

	All HR	<10 BU[a]	≥0 BU	*P*-value
Overall success rate	63%	83%	40%	0.001
Mean time to success (months)	16.3	14.4	21.7	0.02

BU, Bethesda unit.

[a]All 22 HR hemophilia A subjects with pre-ITI titer <2 BU achieved success within a mean treatment time of 7.1 months.

registry, and North American Immune Tolerance Registry (NAITR) have all shown that lower pre-ITI, historical peak and, in the NAITR, ITI peak titers, uniformly and statistically predict ITI success [3,9,10]. Data from the NAITR are shown in Table 10.1. Among host-related variables implicated in FVIII antibody development (Chapter 8), age, ethnicity, and FVIII gene mutations have been examined in relation to ITI outcome. Age at ITI initiation was among the variables for which a discrepant impact on ITI success was reported by the IITR and NAITR [3,10]. The NAITR examined ethnicity in a study cohort demographically representative of the USA, and found ITI success rates among ethnic Africans (67%) and Latinos (58%) to be statistically indistinguishable from that of subjects of other races (71%) [10]. Attempts to correlate specific FVIIII mutations with ITI outcome or time to tolerance have been inconclusive because of small cohort size [11,12]. However, the ongoing I-ITI and Hemophilia Inhibitor Genotype (HIGS) studies both have the potential to further define the role of host factors in ITI outcome.

The role of treatment factors in immune tolerance induction outcome

Delay in the start of immune tolerance induction, treatment interruption

Treatment-associated factors with a potential effect on ITI outcome are summarized in Figure 10.1. Variables that either were not uniformly studied by the registries or for which a discrepant significant effect on ITI success was reported included (i) an interval of over 5 years between inhibitor diagnosis and the start of ITI [3,10] and (ii) an interruption in ITI of over 2 weeks [9].

Factor VIII dose/regimen

As recently summarized, historical reports of an overall similar rate of ITI success (75–90%) among disparate therapeutic regimens first raised the question of the role of FVIII dose on ITI outcome [2]. The IITR did observe that daily FVIII doses of ≥200 units/kg/day resulted in a significantly improved outcome [13]. However, the NAITR reported a significant inverse relationship between FVIII dose and tolerance, although success was achieved more rapidly with doses of ≥50 units/kg/day [10]. Ultimately, a meta-analysis of the two registries determined that, for "good risk" patients with historical inhibitor titers under 200 BU and immediate pre-ITI titers under 10 BU, FVIII dose did not impact ITI outcome [14]. This observation provides the rationale for the ongoing I-ITI study of the impact of FVIII dose on the overall rate of, and time

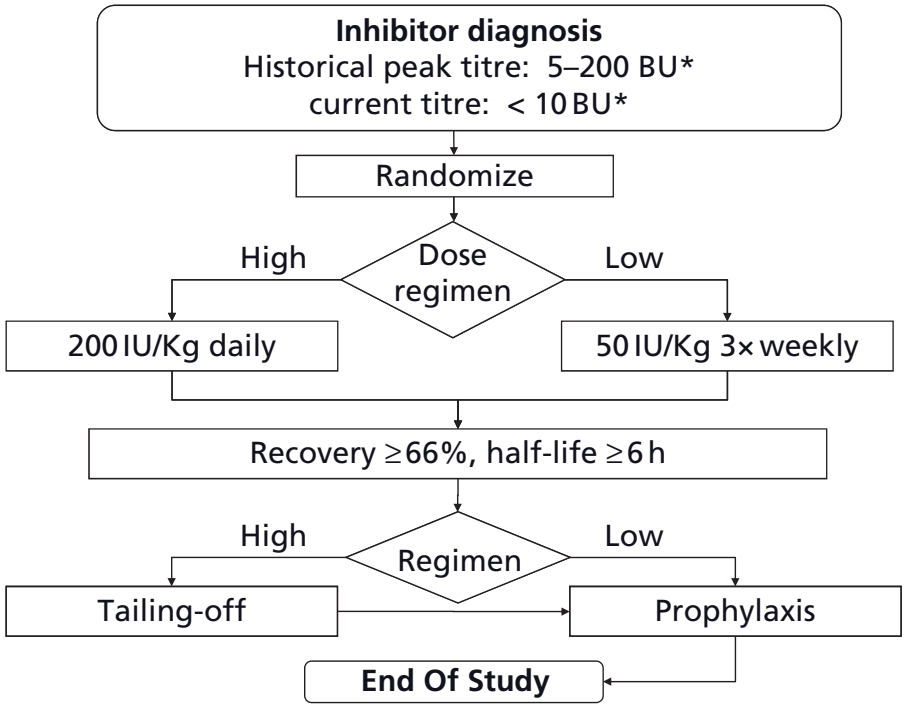

Figure 10.2 Trial design for the International Immune Tolerance Study [5]. BU, Bethesda units.

Table 10.2 Summary of published cohort analyses of product type and purity and ITI outcome [2].

	Study	Product	Dose	Success rate (%)
von Willebrand factor-containing factor VIII	Brackmann (1996) [41]	Haemate P®	High	71–88
	Orsini (2005) [42]	Factane®	Various	87
High-purity plasma-derived/ recombinant factor VIII	Smith (1999) [43]	Monoclonal/recombinant	High	100
	Rocino (2006) [11]	Monoclonal/recombinant	Intermediate	87
Recombinant factor VIII	Lusher (1994) [44]	Kogenate®	High	63
	Bray (1994) [45]	Recombinate®	High	50
	Batlle (1999) [46]	Kogenate®	High	77
	Courter (2001) [47]	Refacto®	High	81
	Barnes (2006) [48]	Kogenate®	Various	76
	Rocino (2001) [49]	Various	Intermediate/high	73

to, ITI success in a cohort of 150 "good risk" severe hemophilia A inhibitor subjects randomized to receive FVIII doses of either 200 units/kg/day or 50 units/kg thrice weekly for up to 33 months (Figure 10.2) [5].

Factor VIII product type and purity

The multiple published reports of successful tolerance induction in "good" and "poor" risk patients with the use of both recombinant and plasma-derived FVIII have been recently summarized (Table 10.2) [2]. However, superior efficacy of one product type was first suggested by a report of a lower ITI success rate (29%) with high-purity FVIII, compared with that historically achieved with intermediate-purity von Willebrand factor (VWF)-containing product (91%) in 14 patients [15]. Neither the IITR nor the NAITR could corroborate this observation because of the skewed distribution of product use in each registry [3,10]. Furthermore, subsequent retrospective reports of institutional [16,17], regional [18], and national [19] experience with ITI salvage therapy using VWF-containing concentrates did not observe similarly high ITI success rates. Nonetheless, there is biochemical rationale for a potential downregulatory effect of VWF on inhibitor development, and, therefore, for the possible positive impact of VWF on ITI outcome [20]. The international prospective randomized RESIST study will attempt to answer this important question in a "poor-risk" hemophilia A cohort (A. Gringeri, Angelo Bianch Bonomi Hemophilila and Thrombosis Centre, Milan, Italy, personal communication).

Immune modulation

Adjunctive use of cyclophosphamide and gammaglobulin formed the cornerstone of the Malmö ITI regimen [21].

However, a subsequent analysis of this protocol demonstrated no outcome advantage of immune modulation (IM) relative to standard ITI [22]. Concerns about long-term cyclophosphamide complications and the technical difficulty of performing extracorporeal immunoadsorption currently limit the use of this strategy in young children.

Renewed interest in IM has focused on selective B-cell depletion using rituximab, a humanized monoclonal antibody to B-cell CD20 antigen. However, the published experience with rituximab for ITI in congenital hemophilia is limited and outcomes are difficult to interpret because of protocol variability and likely positive reporting bias. A recent review calculated initial and long-term success rates of 74% and 53%, respectively, among the 19 cases reported to date, most of which received the standard four-dose regimen, with or without concomitant FVIII therapy [23]. Notably, three of the five reported therapeutic failures did not receive concomitant FVIII ITI [23]. Anticipated side-effects have occurred infrequently among children with hemophilia and other disorders treated with this drug [23]. However, the immediate and long-term complications of anti-CD20 therapy in children are still largely unknown. Most agree that more outcome data are required before this strategy can be widely recommended for children with inhibitors, and would favor prospective data collection through registries and controlled studies. One such study is under way in the USA (C. Leissinger, Tulane University School of Medicine, New Orleans, USA, personal communication).

Supportive care in immune tolerance induction: impact on outcome

Central venous access-associated complications of immune tolerance induction

Given the young age of most ITI patients, adequate venous access is often a crucial component of successful outcome. A European survey of hemophilia centers found that 72% preferred a central venous catheter device (CVAD) for ITI [6]. However, as recently summarized, CVAD complications, particularly infection, adversely affect ITI outcome [24]. Although CVAD infection did not predict ITI outcome in the NAITR, this complication occurred more frequently ($P = 0.0006$) with daily ITI, an observation that was recently corroborated [10,25]. The I-ITI study will generate prospective data on the impact of CVAD complications on ITI outcome [5].

Arteriovenous fistulae (AVF) have been preliminarily reported to be a promising alternative strategy for long-term venous access during ITI without complicating infection [26]. Although this method of venous access may prove to be a safe and effective alternative to the use of CVADs for ITI, several logistic and technical issues must still be identified and resolved before widespread use can be advocated.

Bleeding prophylaxis with bypass therapy

The musculoskeletal complications of inhibitor development have been well documented [27], as has the benefit of primary joint prophylaxis in children without inhibitors (Chapter 5) [28]. Therefore, safe and effective prophylaxis is also the goal for inhibitor patients.

Prophylaxis with an activated prothrombin complex concentrate (aPCC) has historically been a part of the Bonn ITI protocol [1]. Although often implemented in clinical practice, there have been no controlled trials to assess the efficacy and safety of adjunctive bypass therapy prophylaxis during ITI. In one observational study of aPCC prophylaxis (50–200 units/kg daily) in 22 children 6 years undergoing ITI, the median annual incidence of hemarthrosis was 1 (range 0–6) and early radiographic evaluation revealed minimal arthropathy [29]. Thrombosis was not observed, but has since been reported by others [30]. In a recent prospective pilot study of recombinant activated factor VII (rFVIIa) prophylaxis in inhibitor patients not on ITI, bleeding frequency was reduced on both 90 mg/kg and 270 mg/kg daily regimens [31]. Additional prospective data on the safety, efficacy, and cost-efficacy of bypass therapy prophylaxis during ITI as well as on the impact of bleed reduction on ITI outcome will also be forthcoming from the I-ITI study.

Current recommendations for immune tolerance induction in severe hemophilia A

Three independent panels, while advocating for prospective data collection, have developed interim consensus recommendations for the current practice of ITI in patients with severe hemophilia A and high titer (>5 BU) inhibitors, based on a critical review of the existing literature. These include the EHSB and the ICP [6,7] as well as the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) [32]. The ICP recommendations are summarized in Figure 10.3.

All groups agreed that it is preferable to initiate ITI at a titer <10 BU (grade B, level IIB or III) [6,7,32], unless, per the ICP, the titer does not decline over a period of 1–2 years and/or inhibitor development is associated with severe or life-threatening bleeding (grade C, level IV) [7]. The UKHCDO and the ICP also recommended avoidance of FVIII exposure and the use of nonanamnestic bypass therapy for the prevention and treatment of bleeding during the waiting period (grade B, level IIB or III) [7,32].

All three panels also concur that insufficient information exists to make any recommendations about the initial ITI dosing regimen, and that all ITI patients should be enrolled either in a prospective randomized clinical trial or an international registry (grade B or C; level IIB or III) [6,7,32]. The ICP noted that for "poor-risk" ITI patients (defined by a historical

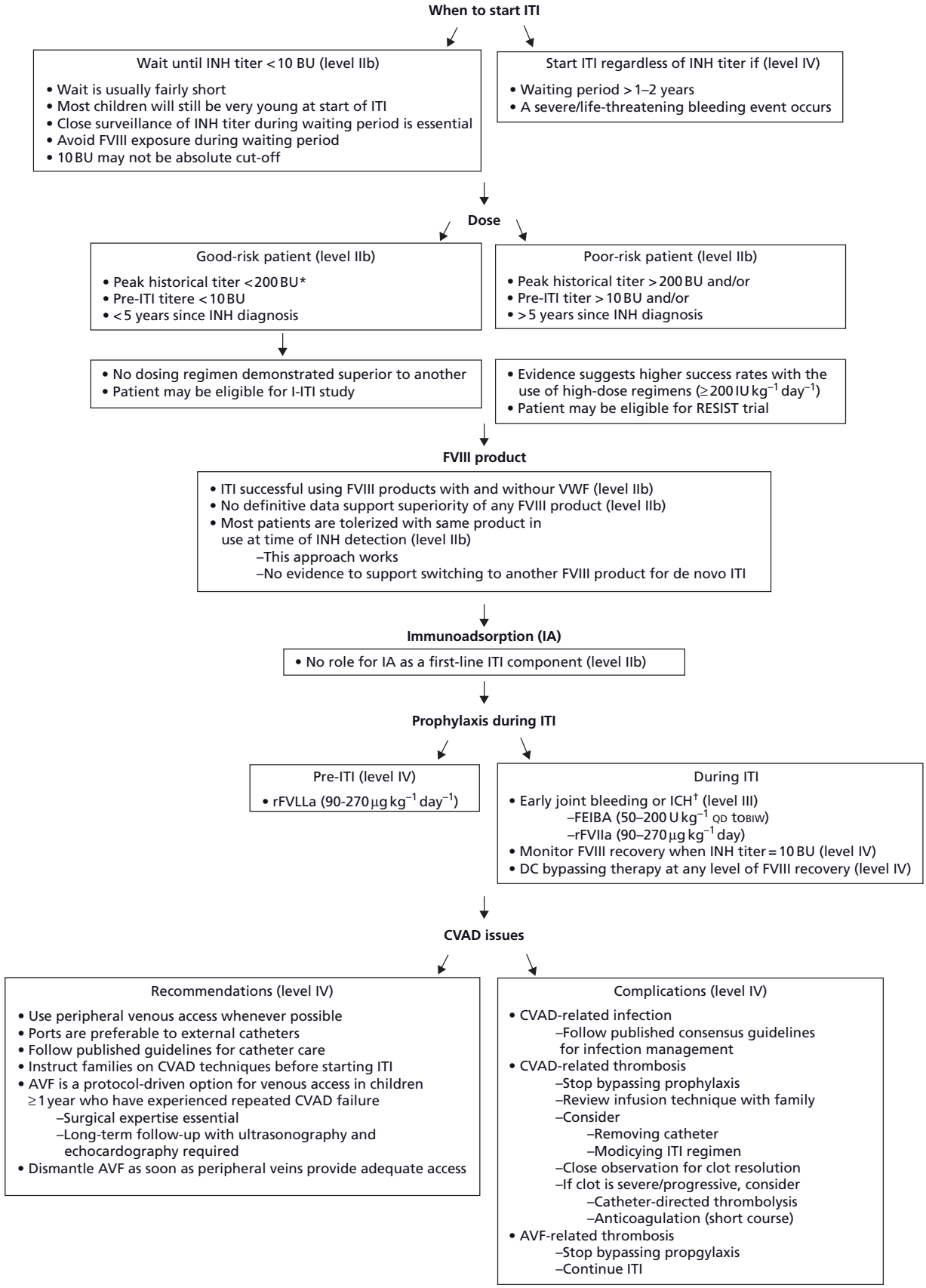

Figure 10.3 International Consensus Panel (ICP) on Immune Tolerance recommendations for immune tolerance of inhibitors and supportive care in severe hemophilia A [10]. *Very good risk patient: peak historic titer <50 BU. †Higher doses used for ICH.

titer of >200 BU and/or a pre-ITI titer of >10 BU and/or an interval of >5 years since inhibitor diagnosis), published efficacy data are limited to dosing regimens of ≥200 U/kg/day (grade C, level III) [7].

The groups all independently concluded that ITI has been successfully performed using recombinant and plasma-derived FVIII replacement therapy (usually the product on which they developed the inhibitor), and that there are no data to support the superiority of any single product type (grade C, level IV) [6,7,32]. However, both EHSB and ICP have suggested that VWF-containing concentrates be considered for patients who fail ITI using high-purity FVIII (grade B, level IIB, III) [6,7].

The EHSB and ICP have further concluded that there is no current role for IM in primary ITI (grade C, level IV) [6,7], but the ICP suggested that rituximab be considered as part of an ongoing clinical trial or registry in cases of ITI failure (grade B, level IIB) [7].

The EHSB also advocated the routine use of CVADs to avoid ITI interruption (grade B, level III) [6]. Conversely, the ICP preferred the use of peripheral venous access, but recommended that, when unavoidable, CVAD use be accompanied by instruction and supervision according to published guidelines (grade B, level III) [7, 24]. The ICP has also recommended that AVF be a protocol-driven time-limited alternative used only in experienced centers for children aged ≥1 year who have experienced repeated CVAD failure (grade B, level III) [7].

Finally, the ICP and EHSB both recommended that bypass therapy prophylaxis be administered at published doses early in the course of ITI when joint or life-threatening bleeding occurs (grade C, level IV) [6,7]. The ICP further recommendeds close monitoring of inhibitor titers and FVIII recovery during ITI, as well as prompt cessation of bypass therapy when a FVIII recovery is established to minimize line-associated thrombosis (grade C, level IV) [7].

Immune tolerance in hemophilia A: special considerations and recommendations

Immune tolerance induction for low-titer/responding inhibitors

Little published information exists on the practice and outcome of ITI in low-titer/responding (LR) hemophilia. A survey of 21 European hemophilia centers revealed that while the majority (17/21) used ITI regimens of ≥100 units/kg/day for high-titer inhibitors, 15/21 routinely used <100 units/kg/day, and 10/21 used <50 units/kg/day regimens for LR inhibitors [6]. No outcome data were reported. The EHSB has recommended that ITI be initiated in children and adults exhibiting persistent LR inhibitors for ≥6 months whose bleeding symptoms cannot be controlled with FVIII replacement (grade C, level IV) [6].

Immune tolerance induction in moderate/mild hemophilia A

Inhibitor development in moderate and mild hemophilia A is discussed in Chapter 9. The limited published ITI experience in moderate/mild hemophilia, largely because of relative infrequency of inhibitors, has recently been summarized [7,33].

The single published cohort study reported a low (25%) success rate with traditional high- and low-dose ITI initiated in eight "poor-risk" adults because of excessive bleeding. Desmopressin has been used for prophylaxis and ITI in cases of non- or low-titer cross-reacting FVIII antibodies. Additionally, traditional immunosuppressive therapy and rituximab have resulted in short-term tolerance in small groups of patients; however, data on FVIII re-challenge is lacking.

Consequently, ICP and UKHCDO recommendations for inhibitor eradication in moderate/mild hemophilia have primarily focused on inhibitor prevention including identification of "at-risk" patients through gene mutation analysis; preferential use of desmopressin therapy when possible; and surveillance for inhibitor development after high-dose FVIII exposure (level IV) [7,32]. Both groups agreed on the potential therapeutic role for traditional ITI and IM for antibodies that fail to spontaneously disappear and/or exhibit anamnesis upon FVIII re-challenge, although the criteria for optimal candidate selection remain unclear (level IV) [7,32]. Finally, both identified the need for an international registry for prospective data collection [7,32].

Immune tolerance: factor IX inhibitors

The historical experience

The epidemiology and treatment of FIX inhibitors is discussed in Chapter 14. Given the low incidence of factor IX inhibitors, the historical experience with immune tolerance in hemophilia B was limited to seven patients for whom the overall success rate with high-dose FIX with or without immune modulation was 71% [1,21]. Long-term ITI success in five of seven hemophilia B patients treated with the Malmö regimen was subsequently reported [22].

The Immune Tolerance Induction Registries: outcome and outcome predictors

The largest collection of data on ITI in hemophilia B derives from the NAITR [10] and the International Factor IX Inhibitor Registry [34]. In the NAITR, only 5/16 (31%) completed courses of ITI in hemophilia B were successful using a median dose of 100 units/kg/day (range 25–200) [10]. Daily dosing regimens and immune modulation were used in 88% and 47%

Table 10.3 Comparison of outcome parameters between hemophilia B subjects successfully and unsuccessfully tolerized within the North American Immune Tolerance Registry (NAITR) [10].

Hemophilia B immune tolerance induction (ITI) outcome	Success (n = 5)	Failure (n = 11)
Factor IX activity <0.01 U/ml	5/5	11/11
High responder (<5 BU)	4/5	9/11
Family history of inhibitor	0	5/11
African-American ethnicity	2/5	2/11
Median age (years) at ITI	3.7	4.6
Median interval inhibitor/ITI (months)	12	47
Median ITI factor IX dose (units/kg/day)	100	75
Median duration of ITI (months)	12	7
Access complications	0/5	9/11
Adverse reactions	2/5	8/11
Allergic reactions	1/5	4/11

BU, Bethesda unit.

of courses, respectively. Plasmapheresis was used in two courses. High-purity or monoclonal FIX concentrates were used 82% of the time [10]. With few data, no association between ITI outcome and FIX dose or purity can yet be established.

The demographics of the NAITR FIX inhibitor relative to outcome are detailed in Table 10.3. Patients with an allergic phenotype in association with FIX antibodies (10/16) were over-represented in this cohort and 80% failed ITI. ITI complications specific to this group of patients may have been responsible for the poor outcome in the entire cohort for which the adverse event rate of 65% was 10-fold higher than that observed for hemophilia A ITI [10]. Reactions to FIX in allergic phenotype patients accounted for 79% of the adverse events [10]. Similarly, poor outcomes (14% of 34 attempts) were reported from the International Factor IX Inhibitor Registry, including no successes among allergic phenotype patients [34].

Nephrotic syndrome as a complication of immune tolerance induction

Nephrotic syndrome represents the most serious complication of ITI in patients with FIX inhibitors associated with allergic manifestations [35]. This complications occurred in 3/10 NAITR FIX inhibitor subjects with this profile [10]. Aggregate data on this phenomenon were also collected through the International Factor IX Inhibitor Registry, in which 11/13 ITI patients who developed nephrotic syndrome had a history of prior anaphylaxis to FIX [34]. Although all FIX product types were implicated, FIX genotypic predilection (deletion or stop codon mutation) was ascertained [34]. The registry documented that the development of periorbital edema, hypoalbuminemia, and proteinuria occurred 8 to 9 months into the course of high-dose ITI (100–325 units/kg/day). Clinical improvement usually followed cessation of FIX, but the response to standard therapy of the renal disease with steroids was poor [34]. So far, the etiology of this complication remains unclear [35].

Immune modulation

Given the poor success rate with ITI in hemophilia B, there has been anecdotal experience with the use of immunomodulatory therapy with mixed success. These reports have recently been summarized [36]. Successful tolerance was induced in a single FIX inhibitor patient using either cyclosporine A or mycophenolate mofetil. Rituximab failed to tolerize two inhibitor patients.

Current recommendations for immune tolerance induction in hemophilia B

Based on this experience, the EHTSB, ICP, and UKHCDO have all concluded that ITI success for FIX inhibitors is low, particularly in patients with the allergic phenotype, and have recommended that attempts at ITI in such patients be cautiously undertaken with close clinical monitoring for early detection of complications, particularly nephrotic syndrome [6,7,32].

Future strategies for immune tolerance induction in hemophilia A and B

Ultimately, FVIII and FIX inhibitor prevention may prove to be the best strategy. Potentially important immunologic mechanisms of tolerance have been studied in animals and corroborated through the *in vitro* study of hemophilic inhibitor plasma [37]. However, these observations now require prospective *in vivo* study in collaboration with clinical ITI trials, and several such efforts are in progress. Furthermore, the immunogenicity of future recombinant or transgenic FVIII and FIX will need to be carefully assessed in preclinical and prelicensure human clinical trials [38]. Finally, the use of gene transfer technology for inhibitor prevention is an intriguing possibility, based on studies conducted in neonatal FIX-deficient mice [39]. Although there were many qualifying aspects to the success of this preliminary work, it represented important proof of principle for this potential strategy. The use of gene transfer technology to downregulate an established antibody remains theoretical. However, successful tolerance of a longstanding high-titer FVIII inhibitor following liver transplantation also provided proof of principle for the potential efficacy of this strategy [40].

Conclusion

Immune tolerance induction has been practiced clinically for 30 years and remains the primary strategy for FVIII and FIX inhibitor eradication. However, as agreed by three independent expert panels, the large body of literature generated by years of international experience with this strategy remains largely unable to provide a strong evidence base for ITI practice recommendations. Generating high-level outcome data for a rare disease is a challenging but plausible mandate for a scientific community with the will to move from an empiric to a collaborative evidence-based approach to the prevention and eradication of FVIII and FIX inhibitors.

References

1 Brackmann HH, Gormsen J. Massive factor-VIII infusion in haemophiliac with factor-VIII inhibitor, high responder. *Lancet* 1977; **2**: 993.

2 DiMichele D. Immune tolerance therapy for factor VIII inhibitors: Moving from empiricism to an evidence-based approach. *J Thromb Haemost* 2007 **5**(Suppl. 1): 143–150.

3 Mariani G, Scheibel E, Nogao T, *et al.* Immune tolerance as treatment of alloantibodies to factor VIII in hemophilia. The International Registry of Immunetolerance Protocols. *Semin Hematol* 1994; **31**(Suppl. 4): 62–64.

4 DiMichele D, Kroner B. Factor VIII/IX Subcommittee of the International Society for Thrombosis and Hemostasis. The maintenance of tolerance after successful immune tolerance induction in haemophilia A and B: The North American Registry. Factor VIII/IX Subcommittee of the International Society for Thrombosis and Haemostasis. *Haematologica* 2000; **85**(Suppl. 10): 40–44.

5 DiMichele DM, Hay CRM. The international immune tolerance study: A multicenter prospective randomised trial in progress. *J Thromb Haemost* 2006; **4**: 2271–3000.

6 Astermark J, Morado M, Rocino A, *et al.* Current European practice in immune tolerance induction therapy in patients with haemophilia and inhibitors. *Haemophilia* 2006; **12**: 363–71.

7 DiMichele DM, Hoots WK, Pipe SW, Rivard GE, Santagostino E. International workshop on immune tolerance induction: Consensus recommendations. *Haemophilia* 2007; **13**: (Suppl. 1): 1–22.

8 vanHelden PMW, van den Berg HM, Gouw SC, *et al.* IgG subclasses of anti-FVIII antibodies during immune tolerance induction in patients with hemophilia A. *Br J Haematol* 2008; **142**: 644–52.

9 Lenk H, ITT Study Group. The German Registry of immune tolerance treatment in hemophilia—1999 update. *Haematologica* 2000; **10**(Suppl.): 45–7.

10 DiMichele DM, Kroner B, and the North American Immune Tolerance Study Group. The North American Immune Tolerance Registry: Practices, outcomes, outcome predictors. *Thromb Haemost* 2002; **87**: 52–57.

11 Rocino A, Santagostino E, Mancuso ME, Mannucci PM. Immune tolerance induction with recombinant factor VIII in haemophilia A patients with high responding inhibitors. *Haematologica* 2006; **91**: 558–61.

12 Oldenburg J, Schwaab R, Brackmann HH. Induction of immune tolerance in haemophilia A. Inhibitor patients by the "Bonn Protocol": Predictive Parameters for Therapy, Duration & Outcome. *Vox Sang* 1999; **77**(Suppl. 1): 49–54.

13 Mariani G, Kroner B. Immune tolerance in haemophilia with factor VIII inhibitors: Predictors of success. *Haematologica* 2001; **86**: 1186–93.

14 Kroner BL. Comparison of the International Immune Tolerance Registry and the North American Immune Tolerance Registry. *Vox Sang* 1999; **77**(Suppl. 1): 33–7.

15 Ettingshausen CE, Kreuz W. Role of von Willebrand factor in immune tolerance induction. *Blood Coagul Fibrinolysis* 2005; **16**: (Suppl. 1): S27–31.

16 Unuvar A, Warrier I, Lusher JM. Immune tolerance induction in the treatment of paediatric haemophilia A patients with factor VIII inhibitors. *Haemophilia* 2000; **6**: 150–7.

17 Greninger DA, Saint-Remy JM, Jacquemin M, Benhida A, DiMichele DM. The use of factor VIII/von Willebrand factor concentrate for immune tolerance induction in haemophilia A patients with high-titre inhibitors: Association of clinical outcome with inhibitor epitope profile. *Haemophilia* 2008; **2**: 295–302.

18 Damiano ML, Hutter JJ, Jr. Immune tolerance for haemophilia patients with inhibitors: Analysis of the western United States experience. The Tri-Regional Nursing Group. *Haemophilia* 2000; **6**: 526–32.

19 Kurth MA, Dimichele D, Sexauer C, *et al.* Immune tolerance therapy utilizing factor VIII/von Willebrand factor concentrate in haemophilia A patients with high titre factor VIII inhibitors. *Haemophilia* 2008; **1**: 50–55.

20 Goudemand J. Inhibitor development in haemophilia A: The role of von Willebrand factor/factor VIII concentrates. *Haemophilia* 2007; **13**(Suppl. 5): 47–51.

21 Nilsson IM, Berntorp E, Zettervall O. Induction of immune tolerance in patients with haemophilia and antibodies to factor VIII by combined treatment with intravenous IgG, cyclophosphamide, and factor VIII. *N Engl J Med* 1988; **318**: 947–50.

22 Freiburghaus C, Berntorp E, Ekman M, Gunnarsson M, Kjellberg BM, Nilsson IM. Tolerance induction using the Malmö treatment model 1982–1995. *Haemophilia* 1999; **5**: 32–9.

23 PW Collins. Novel therapies for immune tolerance in haemophilia A. *Haemophilia* 2006; **12**(Suppl. 6): 94–101.

24 Ewenstein BM, Valentino LA, Journeycake JM, *et al.* Consensus recommendations for use of central venous access devices in haemophilia. *Haemophilia* 2004; **10**: 629–48.

25 Mancuso ME, Mannucci PM, Sartori A, Agliardi A, Santagostino E. Feasibility of prophylaxis and immune tolerance induction regimens in haemophilic children using fully implantable central venous catheters. *Br J Haematol* 2008; **141**: 689–95.

26 Santagostino E, Gringeri A, Berardinelli L, Beretta C, Muca-Perja M, Mannucci PM. Long-term safety and feasibility of arteriovenous fistulae as vascular accesses in children with haemophilia: A prospective study. *Br J Haematol* 2003; **123**: 502–6.

27 Soucie JM, Cianfrini C, Janco RL, *et al.* Joint-range-of motion limitations among young males with haemophilia: Prevalence and risk factors. *Blood* 2004; **103**: 2467–73.

28 Manco-Johnson ML, Funk S, Jacobson L, *et al.* Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; **357**: 535–44.

29 Kreuz W, Escuriola-Ettingshausen C, Mentzer D, *et al.* Factor VIII inhibitor bypass activity (FEIBA) for prophylaxis during immune tolerance induction (ITI) in patients with high-responding inhibitors [Abstract]. *Blood* 2000; **96**: 266a. Abstract 1141.

30 Carcao MD, Connolly BL, Chait P, *et al.* Central venous catheter-related thrombosis presenting as superior vena cava syndrome in

a haemophilic patient with inhibitors. *Haemophilia* 2003; **5**: 578–83.
31 Konkle BA, Ebbesen LS, Erhardtsen E, *et al.* Randomised, prospective clinical trial of recombinant factor VIIa for secondary prophylaxis in hemophilia patients with inhibitors. *J Thromb Haemost* 2007; **9**: 1904–13.
32 Hay CRM, Brown S, Collins PW, Keeling DM, Liesner R. The diagnosis and management of factor VIII and IX inhibitors: A guideline from the United Kingdom Centre Doctors Organisation. *Br J Haematol* 2006; **133**: 591–605.
33 Hay CR, Ludlam CA, Colvin BT, *et al.* Factor VIII inhibitors in mild and moderate-severity haemophilia A. UK Haemophilia Centre Directors Organisation. *Thromb Haemost* 1998; **79**: 762–6.
34 Warrier I. Inhibitors in haemophilia B. In: Lee C, Berntorp E, Hoots KW (eds.) *Textbook of Hemophilia*. Oxford: Blackwell Publishers, 2005; 97–100
35 Ewenstein BM, Takemoto C, Warrier I, *et al.* Nephrotic syndrome as a complication of immune tolerance in hemophilia B. *Blood* 1997; **89**: 1115–16.
36 DiMichele D. (2008) The North American immune tolerance registry: Contributions to the thirty-year experience with immune tolerance therapy. *Haemophilia* 2009; **15**: 320–8.
37 Reipert BM, van den Helden PMW, Schwarz HP, Causl C. Mechanisms of action of immune tolerance induction against factor VIII in patients with congenital haemophilia A and factor VIII inhibitors. *Br J Haematol* 2006; **136**: 12–25.
38 Pipe SW. The promise and challenges of bioengineered recombinant clotting factors. *J Thromb Haemost* 2005; volume: 1692–701.
39 Dobrzynski E, Fitzgerald JC, Cao O, Mingozzi F, Wang L, Hertzog RW. Prevention of cytotoxic T lymphocyte responses to factor IX-expressing hepatocytes by gene transfer-induces regulatory T cells. *Proc Natl Acad Sci USA* 2006; **103**: 4592–7.
40 Ashrani AA, Reding MT, Shet A, *et al.* Successful liver transplantation in a patient with severe haemophilia A and a high titre factor VIII inhibitor. *Haemophilia* 2004; **10**: 735–7.
41 Brackmann HH, Oldenburg J, Schwaab R. Immune tolerance for the treatment of factor VIII inhibitors—twenty years' 'bonn protocol'. *Vox Sang* 1996; **70**(Suppl. 1): 30–35.
42 Orsini F, Rothschild C, Beurrier P, Faradji A, Goudemand J, Polack B. Immune tolerance induction with highly purified plasma-derived factor VIII containing von Willebrand factor in hemophilia A patients with high-responding inhibitors. *Haematologica* 2005; **90**: 1288–90.
43 Smith MP, Spence KJ, Waters EL, *et al.* Immune tolerance therapy for hemophilia A patients with acquired factor VIII alloantibodies: comprehensive analysis of experience at a single institution. *Thromb Haemost* 1999; **81**: 35–88.
44 Luster JM. Summary of clinical experience with recombinant factor VIII products—Kogenate. *Ann Hematol* 1994; **68**(Suppl. 3): S3–6.
45 Bray GL, Gomperts ED, Courter S, *et al.* A multicenter study of recombinant factor VIII (Recombinate): safety, efficacy, and inhibitor risk in previously untreated patients with hemophilia A. The Recombinate Study Group. *Blood* 1994; **83**: 2428–35.
46 Batlle J, Lopez MF, Brackmann HH. et al. Induction of immune tolerance with recombinant factor VIII in haemophilia A patients with inhibitors. *Haemophilia* 1999; **5**: 431–5.
47 Courter SG, Bedrosian CL. Clinical evaluation of B-domain deleted recombinant factor VIII in previously untreated patients. *Semin Hematol* 2001; **38** (Suppl. 4): 52–9.
48 Barnes C, Rivard GE, Poon MC, *et al.* Inhibitor Subcommittee of Association of Heuophilia Clinic Directors of Canada. Canadian multi-institutional survey of immune tolerance therapy (ITT)—experience with the use of recombinant factor VIII for ITT. *Haemophilia* 2006; **12**: 1–6.
49 Rocino A, Papa ML, Salerno E, Capasso F, Miraglia E, deBiasi R. Immune tolerance induction in hemophilia A patients with high-responding inhibitors to factor VIII: experience at a single institution. *Haemophilia* 2001; **7**: 33–8.

11 Inhibitors to factor VIII: treatment of acute bleeds

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The treatment of acute bleeds in patients who have developed inhibitors of factor VIII (FVIII) has benefited in the last decades from the development of new drugs, which has dramatically improved the prognosis of patients who experience such events. Several therapeutic agents are available to treat bleeds in hemophiliacs with inhibitors, but no single agent is efficacious in all patients or all circumstances. Although the management of patients with high-responding inhibitors is still complex and remains a medical challenge in some clinical situations, these products may not only ameliorate the pain, but also reduce the risk of muscular and skeletal damage. They also improve the educational and work prospects for these patients as well as enhancing their social participation and quality of life. However, an increased morbidity is associated with these acute bleeds that require an early treatment with the most adapted concentrate.

Clinical context

Acute bleeds may occur in various clinical contexts, but two characteristics influence product choice to achieve hemostasis. On the one hand, patients with low- or high-titer inhibitors are considered to exhibit the bleeding profile of a severe hemophiliac. This means that they suffer two to four bleeding episodes per month, although some patients demonstrate a decrease in the frequency of bleedings with aging. Most of these bleeds occur spontaneously (in everyday life) and will be successfully treated with home treatment. In contrast, some acute bleeds may be life threatening or may arise in critical situations such as perioperative periods. Although both types of clinical settings represent acute conditions, the nature of treatment to be administered may be different, depending on the titer of the inhibitor at that moment (Figure 11.1).

Classification between high and low responders

Inhibitors have been classified according to peak historical antibody titer and the presence or absence of immunologic anamnesis. A recent consensus of the FVIII and FIX Standardization Subcommittee of the International Society of Thrombosis and Hemostasis defined high-responding antibodies as those exhibiting a peak historical titer of >5 Bethesda units (BU) accompanied by brisk anamnesis and the consequent inability to treat hemorrhages routinely with specific factor replacement [1]. Accordingly, a low responder was defined by both a low historical peak titer (<5 BU) and a lack of anamnesis upon factor re-exposure. As a consequence, these latter patients can usually be treated with higher than usual doses of specific clotting factor concentrate to override the inhibitory effect of the antibody.

Products available

The available therapeutic agents for treatment of acute hemorrhage in hemophiliacs with an inhibitor include high-dose human FVIII concentrate, porcine FVIII concentrate, activated prothrombin complex concentrates (aPCCs), and recombinant FVIIa (rFVIIa). In addition, antifibrinolytics and external removal of the inhibitory antibodies may be used as an adjunct therapy.

Human factor VIII concentrates

For low-titer inhibitors (<5 BU), high doses of FVIII concentrates may be used to achieve hemostasis [2]. Both plasma-derived and rFVIII can be administered with no objective difference in terms of *in vivo* efficacy between these types of products. The dose is targeted to saturate the inhibitor (number of units to be infused = plasma volume in mL × inhibitor titer in BU/mL) and to raise the FVIII plasma concentration to the desired level after inhibitor neutralization [considering a mean recovery of 2 U/dL/IU infused/kg body weight (b.w.)]. The major advantages of this modality are achievement of a predictable hemostasis according to the plasma level achieved and the ability to undertake biologic monitoring using standard coagulation techniques, if needed. The main disadvantage of this strategy is the subsequent anamnestic response that may occur in some patients, which may decrease the possibility of further use in the next months or years. In general, inhibitors of more than 10 BU cannot be saturated with high-dose FVIII;

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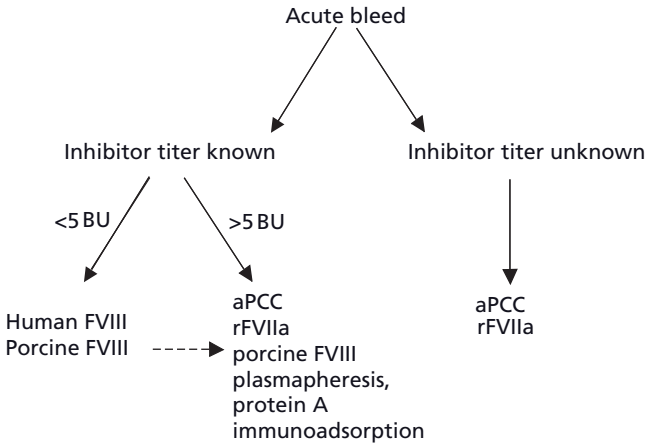

Figure 11.1 Schematic diagram of the therapeutic options for the treatment of acute bleeds according to the inhibitor titer (in Bethesda units/mL).

further, the clinical benefit is not predictable when the titer is between 5 and 10 BU.

Porcine factor VIII

High-purity porcine FVIII (Hyate:C, Ipsen, UK) was developed on the premise that human FVIII inhibitors have a median 15–30% cross-reactivity to porcine FVIII [3]. Porcine FVIII has the same procoagulant activity as human FVIII and is used for treating serious bleeding in patients with low- or intermediate-level inhibitor titers against the porcine FVIII molecule. Well-selected patients may achieve hemostasis in 80–90% of bleeding episodes, considering a mean recovery of 1.3 U/dL/IU injected/kg b.w. [4,5]. The administration of porcine FVIII may be associated with a transient fall in platelet count and allergic reactions in some individuals, partly because of the presence of high-molecular-weight multimers of porcine von Willebrand factor. An anamnestic response commonly occurs in 50–70% of high responders following the infusion of the porcine molecule. This may render the patient completely refractory to further administration for a protracted period of time.

Plasma-derived porcine FVIII was not virally attenuated, although it has not been shown to transmit any viral disease to human recipients. However, the demand for improved side-effect profile and viral safety has driven the development of a recombinant porcine product which will be highly purified and will include two viral reduction/inactivation steps. This B-domain-deleted recombinant porcine FVIII molecule is currently under clinical evaluation.

Activated prothrombin complex concentrates

Activated prothrombin complex concentrates (aPCCs) represented a considerable improvement in inhibitor therapy when they appeared in the 1970s [6]. These products were established as standard first-line treatment for bleeding episodes in high-titer inhibitors. Two aPCCs were produced by *in vitro* modification of plasma-derived prothrombin complex concentrates (PCCs), resulting in a degree of activation of some of the clotting factors. They contained multiple activated serine protease molecules, of which activated factor X (FX) and prothrombin represent the main active components in FEIBA (Factor Eight Inhibitor Bypassing Activity; Baxter Healthcare Corporation Glendale, CA, USA), while in Autoplexâ-T (Nabi Biopharmaceuticals, Boca Raton, FL, USA), FIX and FVII are thought to be the putative active hemostatic proteases [7,8]. However, the precise mechanism of action of these aPCCs remains poorly understood. This likely explains the difficulty of a standardized therapeutic follow-up *in vivo*.

Table 11.1 Summary of FEIBA efficacy for treatment of various bleeding events (both home and hospital treatments).

Reference	Patients	Bleeding episodes	Dose of Autoplex/ FEIBA	Efficacy (%) (time for evaluation)
White [14]	23	54	75 FECU/kg 8- to 12-hourly	85 (72 h)
Sjamsoedin *et al.* [9]	15	150	88 U/kg	64 (24 h)
Hilgartner and Knatterud [12]	49	165	50–70 U/kg 12-hourly	91 (72 h)
Hilgartner *et al.* [13]	41	106	50–75 U/kg 12-hourly	79 (36 h)
Negrier *et al.* [10]	60	433	65–100 U/ kg 6- to 12-hourly	81 (up to three doses)
Dimichele and Negrier [11]	169	49 (acute bleeds)	10–147 U/ kg 6- to 24-hourly	82

Kurczynski and Penner [6] reported in 1974 the first significant study on the use of Autoplex®-T. Single doses of Autoplex®-T were used to treat 60 bleeds in eight severe hemophilia A inhibitor patients. The production of this product has, however, been discontinued and yet only one aPCC (FEIBA) is available.

Sjamsoedin *et al.* [9] studied the effect of FEIBA on joint and muscle bleeding in hemophilia A inhibitor patients (Table 11.1). This randomized double-blind trial compared FEIBA with a nonactivated PCC. Outcome comparison showed that FEIBA produced significantly greater improvements in bleeding control and joint mobility. The largest observational trial on the use of FEIBA was a retrospective multicenter French study [10]. Data were presented on 433 bleeding episodes, including surgical procedures involving 60 patients. Efficacy was good or excellent in 81.3% of treatment episodes, while tolerance (safety) was assessed as good in 98.8% of the cases. In an international retrospective postlicensure survey, data

Table 11.2 Summary of home treatment—minor procedures performed with recombinant factor VIIa.

Study	Number of cases	Number of bleeds	Dose (μg/kg)	Achievement of hemostasis	Adverse effects	Mean number of injections
Key *et al.* [20]	60	614	90	566 (92%)	32 (3%)	2.2
Laurian *et al.* [21]	147	16	90–120	90%	1 (0.6%)	3.8
Santagostino *et al.* [22]	21	53	90	42 (79%)	3 (2.6%)	2

were collected on 63 patients with inhibitors (60 patients with hemophilia A; three patients with hemophilia B). Efficacy was reported good or excellent in 82% of all acute episodes, and 91% of all surgical treatments [11].

In summary, it can be estimated that both products have been found to achieve effective hemostasis in approximately 80% of bleeds involving joints and soft tissues [9–14].

Both FEIBA and Autoplex®-T underwent viral inactivation procedures during their manufacturing processes. A second virus inactivation step has been implemented recently for FEIBA production. Minor reactions have been reported in association with both products, mainly consisting of headache, nausea, pruritus, skin rashes, and diarrhea. Anamnestic increases in inhibitor levels have been reported in up to 30% of patients receiving FEIBA, owing to the presence of small amounts of FVIII:C antigen in the material. However, it was shown that in over 50% of the patients who remain on regular FEIBA therapy, the antibody level gradually falls [10]. However, anamnestic responses to aPCC are not associated with a reduction in their clinical efficacy.

For treatment of acute bleeds, the recommended dose of FEIBA is 50–100 U/kg infused every 8–12 h. Because of the potential for thrombotic complications, a maximum daily dose of 200 U/kg is recommended.

Despite many years of experience with these concentrates, there is currently no laboratory test widely available for monitoring the hemostatic efficacy of aPCC, and dosage is often determined solely by clinical assessment. Future studies will be needed to demonstrate the clinical usefulness of a monitoring system, which would correlate with hemostatic efficacy. However, it was recently proposed that the clinical effect of FEIBA may correlate with circulating thrombin-generating potential, and a fluorogenic substrate assay is being developed to enable monitoring of FEIBA dosage *in vivo* [15].

Recombinant factor VIIa

Recombinant factor VIIa (rFVIIa) (NovoSeven®, NovoNordisk, Bagsvaerd, Denmark) is produced as a single-chain glycoprotein in genetically modified BHK (baby hamster kidney) cell line. During purification, rFVII is converted to the two-chain activated form. The mode of action of rFVIIa may not be completely understood, but it has been demonstrated that rFVIIa is able to directly activate FX and increase thrombin production on the surface of activated platelets in the absence of FVIII or FIX. The platelet-specific generation of IIa by rVIIa is thought to localize the hemostatic process to the sites of active bleeding and tissue injury [16,17]. The pharmacokinetic evaluation of rFVIIa demonstrated that the half-life is 2–3 h in adults and 1.5–3 h in children with individual variations [18]. Owing to these pharmacokinetic properties, rFVIIa needs to be administered every 2–6 h. The first description of clinical efficacy occurred in 1988 in a patient with high-titer inhibitor who underwent an open synovectomy [19]. As shown in Table 11.2, initial clinical experience has shown that the treatment of home bleeds usually required a median of more than two rFVIIa injections of 70–90 μg/kg [20–22]. To improve the clinical efficacy of treatment with rFVIIa in high-titer inhibitor patients, attention must be paid to the time of initiating treatment after the onset of bleeding. Early injection of rFVIIa within 1 h following the onset of bleeding has been associated with a better clinical outcome [23].

A few case reports have suggested the possibility that higher rFVIIa doses might be more convenient, and perhaps more efficacious, for the treatment of bleeding episodes. It is suggested that a full thrombin burst is necessary for the assembly of a tight fibrin structure that efficiently stabilizes the hemostatic plug [24] and induces a complete activation of other clotting factors, including the thrombin-activatable fibrinolytic inhibitor (TAFI) [25]. A first prospective and comparative evaluation of high bolus doses of rFVIIa, i.e., 300 μg/kg, was reported by Kenet *et al.* [26] in three patients with high-titer inhibitors. Pain relief was faster and treatment duration was shorter for the bleeding episodes treated by the megadose protocol. In addition, this increased dose was found to be more convenient and the rFVIIa total consumption per bleeding episode was also significantly lower. No thrombosis was reported in the three patients. The fact that hemostasis was achieved in all patients with a single (83% success rate) or two (100% success rate) injections suggests that this mode of delivering rFVIIa might be more efficacious than with lower doses as previously observed [27]. Additional information was provided by the results from two recent clinical trials that demonstrated that rFVIIa was indeed safe and efficacious at doses higher than 90 μg/kg. Santagostino *et al.* [28] reported on a multicenter randomized open-label crossover prospective

trial designed to compare the efficacy, safety of standard and high dosages of recombinant factor VIIa for home treatment of hemarthroses in hemophiliacs with inhibitors. They found that success rates for standard- and high-dose regimens were similar. In another multicenter, randomized, crossover, double-blind trial, patients were randomly allocated to treat a first joint bleeding episode with one 270 μg/kg rFVIIa dose followed by two doses of placebo at 3-h intervals and a second joint bleed with three single doses of 90 μg/kg rFVIIa at 3-h intervals, or vice versa. Both dosages were found similarly effective, and no safety issues were identified [29].

As no FVIII is contained in the product, there is no risk of anamnestic response. However, it should be emphasized that, analogous to aPCC, there is as yet no standardized quantitative laboratory test for measuring the effectiveness of rFVIIa therapy. However, promising results have recently been reported using thromboelastography and thrombin generation [30,31].

All currently used bypassing agents (PCCs, aPCC, and rFVIIa) for the treatment of patients with high-titer inhibitory antibodies carry the risk of thrombotic complications, including thromboembolism, disseminated intravascular coagulation, and myocardial infarction [32,33]. These complications are very rare in hemophilia population and are considered to be caused by an increase in the concentration of native, or activated, coagulation factors or excessive tissue factor release in the recipient with preexisting conditions [34–37]. Thrombotic events (e.g., cardiovascular and cerebrovascular) and subclinical disseminated intravascular coagulation (DIC) occur most commonly in patients with underlying atherosclerotic disease and those immobile for long periods. Patients with pre-existing liver disease and premature infants may be particularly susceptible to DIC. In addition, patients undergoing surgery or those who receive repeated high doses of the concentrate should be carefully monitored to minimize the risk of thrombogenicity. Finally, since the life expectancy of the patients with hemophilia and inhibitors is increasing, one might expect that thrombotic challenges coming with age, particularly on the arterial side, may arise more frequently and require specific attention when bypassing agents are administered sometimes in association with antiplatelet drugs.

Antifibrinolytics

Tranexamic acid is a structural analog that binds irreversibly to the lysine-binding sites on plasminogen, thus inhibiting fibrinolysis. Through the inhibition of the natural degradation of fibrin, it helps to stabilize clots. Although they have a short plasma half-life (~2 h), antifibrinolytic agents may be helpful in the treatment of bleeding from the gastrointestinal tract, menorrhagia, epistaxis, and oral bleeding (including in dental surgery) in inhibitor-developing hemophiliacs [38,39]. It can be administered orally or intravenously, or as a mouthwash. Dosing of tranexamic acid must be reduced in patients with renal dysfunction; further, it is generally advisable not to use tranexamic acid or any other antifibrinolytic drug, including epsilon-aminocaproic acid (Amicar®), for the treatment of hematuria because blood from the upper urinary tract can provoke painful clot retention. Their use during surgery is still a matter of debate, though their utilization tends to increase.

Immunoadsorption

Plasmapheresis or immunoadsorption of plasma with staphylococcal protein A removes immunoglobulins and immune complexes, including inhibitors to FVIII. Although apheresis or immunoadsorption instruments are relatively complex and expensive, the procedure may be cost-effective, given the expense of therapies used to treat patients with inhibitors, particularly in an acute surgical or life-threatening situation. If the inhibitory antibodies are effectively removed, the decrease in the inhibitor titer may render the patient susceptible to be treated by human or porcine FVIII concentrates during 3–6 days before the anamnestic response occurs [40–43].

Management of bleeding situations

The management of bleeding episodes in patients with inhibitors may take advantage of the differential use of the various therapeutic approaches mentioned above. No single product may meet all clinical requirements. The most applicable strategy depends on clinical assessment of severity, knowledge of inhibitor level to human and porcine FVIII and, if the titer is low, whether the patient is a high or a low responder [44,45].

One should therefore consider different clinical scenarios:

- Scenario 1: in low-titer and low responders, it seems logical to preferentially use higher than normal doses of human FVIII in all clinical situations. The dose of FVIII is increased proportionately to the inhibitor titer. For major hemorrhages, this strategy can also be used if inhibitor titers are low enough to allow satisfactory plasma levels to be achieved. When recombinant porcine FVIII will be again available, it could also be prescribed in limb- or life-threatening bleeds in which there is no significant product cross-reactivity with the patient's inhibitor. Antifibrinolytic agents are useful as local administration such as mouthwash or systemically.
- Scenario 2: in low-titer and high responders one would favor rFVIIa or aPCCs for minor bleeding considering the high efficiency of those products. This strategy allows one to reserve FVIII for further treatment of more critical situations. For major bleeds, high-dose human (or porcine) FVIII concentrate is able to produce effective hemostasis, but it should be recognized that the consequence of such treatment will be an almost systematic anamnestic response to FVIII molecule after 3–7 days of treatment. This subsequent increase in the inhibitor titer commonly renders the patient refractory to the con-

tinuation of factor replacement, although concomitant antibody removal using plasmapheresis or protein A adsorption may be attempted. It is common that the inhibitor titer remains above 5 BU/mL for weeks or months, during which time the patient cannot be treated with FVIII. In this situation, a second-line therapy with aPCC or rFVIIa has to be considered if the bleeding complication needs further treatment.

- Scenario 3: in a patient known to be a high responder with a high-titer inhibitor (>5 BU), minor bleeding may usually be treated with aPCC or rFVIIa (one to four injections as necessary). The treatment of major bleeding in this category of patients represents one of the most challenging situations in the treatment of patients with hemophilia. Considering that an ability to monitor *in vivo* FVIII levels confers predictable correlation with physiologic concentrations, porcine FVIII (when available) may be used as first-line therapy if the antiporcine FVIII inhibitor level is below 5 or 10 BU, though good clinical response has been observed in patients with higher titers. If antiporcine inhibitor titers are high or unknown, rFVIIa or an aPCC is preferred. However, the efficacy of each bypassing agent can vary, and neither agent is universally effective [46]. The reasons for such variability have yet to be confirmed, but may involve patient-specific factors, the mechanisms of action and the pharmacokinetic profiles of these two agents. In the case of failure to control the bleeding, sequential administration of rFIIa and FEIBA has been successfully described in case series [47]. Alternatively, immunoadsorption may temporarily reduce the inhibitor, enabling replacement therapy with FVIII for several days. Antifibrinolytic agents can also be used if there are no contraindications (see above).

Conclusion

The management of acute bleeds in patients who have developed inhibitory antibodies remains a medical challenge for those treating hemophilia, particularly in critical medical conditions. Although the administration of human or porcine factor VIII is generally preferred when the inhibitor titer is <5 BU, the anamnestic response may render the patient completely refractory to the clotting factor. Bypassing agents have dramatically improved the home treatment setting, but the impracticality of monitoring for efficacy and the lack of a predictable clinical dose–response relationship represents a major limitation. However, some preliminary positive results using thrombin-generating potential or thrombelastographic analysis of whole blood clotting may prove their value in the near future for guiding clinical practice. This is also an active field of investigation in terms of future therapeutic molecules, and recombinant porcine factor VIII, rFVIIa with improved hemostatic efficacy owing to specific modifications of the molecule should further improve the clinical outcome of these patients.

References

1 White GC, 2nd, Rosendaal F, Aledort LM, *et al.* Factor VIII and factor IX Subcommittee. Definitions in hemophilia. Recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International Society on Thrombosis and Haemostasis. *Thromb Haemost* 2001; **85**: 560.

2 Kasper CK. Human factor VIII for bleeding in patients with inhibitors. *Vox Sang* 1999; **77**(Suppl. 1): 47–8.

3 Kernoff PB. Rationale and evolution of therapy with porcine factor VIII:C. *Am J Med* 1991; **91**: 20–2.

4 Brettler DB, Forsberg AD, Levine PH, *et al.* The use of porcine factor VIII concentrate (Hyate C) in the treatment of patients with inhibitor antibodies to factor FVIII: A multicenter US experience. *Arch Intern Med* 1989; **149**: 1381–5.

5 Hay CR, Lozier JN, Lee CA, *et al.* Safety profile of porcine factor VIII and its use as hospital and home therapy for patients with haemophilia A and inhibitors: The results of an international survey. *Thromb Haemost* 1996; **75**: 25–9.

6 Kurczynski EM, Penner JA. Activated prothrombin concentrate for patients with factor VIII inhibitors. *N Engl J Med* 1974; **291**: 164–7.

7 Turecek PL, Varadi K, Gritsch H, *et al.* Factor Xa and prothrombin: Mechanism of action of FEIBA. *Vox Sang* 1999; **77**(Suppl. 1): 72–9.

8 Lundblad RL, Bergstrom J, De Vreker R, *et al.* Measurement of active coagulation factors in Autoplex®-T with colorimetric active site-specific assay technology. *Thromb Haemost* 1998; **80**: 811–15.

9 Sjamsoedin LJ, Heijnen L, Mauser-Bunschoten EP, *et al.* The effect of activated prothrombin-complex concentrate (FEIBA) on joint and muscle bleeding in patients with haemophilia A and antibodies to factor VIII. A double-blind clinical trial. *N Engl J Med* 1981; **305**: 717–21.

10 Negrier C, Goudemand J, Sultan Y, *et al.* and the members of the French FEIBA Study Group. Multicenter retrospective study on the utilization of FEIBA in France in patients with factor VIII and factor IX inhibitors. *Thromb Haemost* 1997; **77**: 1113–19.

11 Dimichele D, Négrier C. A retrospective postlicensure survey of FEIBA efficacy and safety. *Haemophilia* 2006; **12**: 352–62.

12 Hilgartner MW, Knatterud GL. The use of factor eight inhibitor by-passing activity (FEIBA immuno) product for treatment of bleeding episodes in haemophiliacs with inhibitors. *Blood* 1983; **61**: 36–40.

13 Hilgartner M, Aledort L, Andes A, Gill J. Efficacy and safety of vapor-heated anti-inhibitor coagulant complex in hemophilia patients. FEIBA Study Group. *Transfusion* 1990; **30**: 626–30.

14 White GC 2nd. Seventeen years' experience with Autoplex/Autoplex T: Evaluation of inpatients with severe haemophilia A and factor VIII inhibitors at a major haemophilia centre. *Haemophilia* 2000; **6**: 508–12.

15 Varadi K, Negrier C, Berntorp E, *et al.* Monitoring the bioavailability of FEIBA with a thrombin generation assay. *J Thromb Haemost* 2003; **11**: 2374–80.

16 Monroe DM, Hoffman M, Oliver JA, Roberts HR. Platelet activity of high dose factor VIIa is independent of tissue factor. *Br J Haematol* 1997; **99**: 542–7.

17 Hoffman M, Monroe DM III, Roberts HR. Activated factor VII activates FIX and FX on the surface of activated platelets: Thoughts on the mechanism of action of high dose activated factor VII. *Blood Coagul Fibrinolysis* 1998; **9**: S61–S65.

18 Lindley CM, Sawyer WT, Macik BG, *et al.* Pharmacokinetics and pharmacodynamics of recombinant factor VIIa. *Clin Pharmacol Ther* 1994; **55**: 638–48.
19 Hedner U, Glazer S, Pingel K, *et al.* Successful use of recombinant factor VIIa in patient with severe haemophilia A during synovectomy. *Lancet* 1988; **2**: 1193.
20 Key NS, Aledort LM, Beardsley D, *et al.* Home treatment of mild to moderate bleeding episodes using recombinant factor VIIa (Novoseven) in haemophiliacs with inhibitors. *Thromb Haemost* 1998; **80**: 912–18.
21 Laurian Y, Goudemand J, Negrier C, *et al.* Use of recombinant activated factor VII as first line therapy for bleeding episodes in hemophiliacs with factor VIII or IX inhibitors (NOSEPAC study). *Blood Coagul Fibrinolysis* 1998; **9**: S155–S156.
22 Santagostino E, Gringeri A, Mannucci PM. Home treatment with recombinant activated factor VII in patients with factor VIII inhibitors: The advantages of early intervention. *Br J Haematol* 1999; **104**: 22–6.
23 Lusher JM. Acute hemarthroses: The benefits of early versus late treatment with recombinant activated factor VII. *Blood Coagul Fibrinolysis* 2000; **11**: S45–S49.
24 Blomback B, Carlsson K, Fatah K, *et al.* Fibrin in human plasma gel architectures governed by rate and nature of fibrinogen activation. *Thromb Res* 1994; **75**: 521–38.
25 Lisman T, Mosnier LO, Lambert T, *et al.* Inhibition of fibrinolysis by recombinant factor VIIa in plasma from patients with severe hemophilia A. *Blood* 2002; **99**: 175–9.
26 Kenet G, Lubetsky A, Luboshitz J, Martinowitz U. A new approach to treatment of bleeding episodes in young hemophilia patients: A single bolus megadose of recombinant activated factor VII (NovoSeven). *J Thromb Haemost* 2003; **1**: 450–55.
27 Lusher J, Ingerslev J, Roberts HR, Hedner U. Clinical experience with recombinant factor VIIa: A review. *Blood Coagul Fibrinolysis* 1998; **9**: 119–28.
28 Santagostino E, Mancuso ME, Rocino A, Mancuso G, Scaraggi F, Mannucci PM. A prospective randomized trial of high and standard dosages of recombinant factor VIIa for treatment of hemarthroses in hemophiliacs with inhibitors. *J Thromb Haemost* 2006; **4**: 367–71.
29 Kavakli K, Makris M, Zulfikar B, Erhardtsen E, Abrams ZS, Kenet G; NovoSeven trial (F7HAEM-1510) investigators. Home treatment of haemarthroses using a single dose regimen of recombinant activated factor VII in patients with haemophilia and inhibitors. A multi-centre, randomised, double-blind, cross-over trial. *Thromb Haemost* 2006; **95**: 600–5.
30 Sorensen B, Ingerslev J. Whole blood clot formation phenotypes in hemophilia A and rare coagulation disorders. Patterns of response to recombinant factor VIIa. *J Thromb Haemost* 2004; **2**: 102–10.
31 Dargaud Y, Bordet JC, Lienhart A, Negrier C. Use of the thrombin generation test to evaluate response to treatment with recombinant activated factor VII. *Semin Hematol* 2008; **45**: S72–3.
32 Kohler M. Thrombogenicity of prothrombin complex concentrates. *Thromb Res* 1999; **95**: S13–S17.
33 Peerlinck K, Vermylen J. Acute myocardial infarction following administration of recombinant activated factor VII (Novo seven) in a patient with haemophilia A and inhibitor. *Thromb Haemost* 1999; **82**: 1775–6.
34 Mizon P, Goudemand J, Jude B, Marey A. Myocardial infarction after FEIBA therapy in a hemophilia-B patient with a factor IX inhibitor. *Ann Hematol* 1992; **64**: 309–11.
35 Roberts HR. Clinical experience with activated factor VII: Focus on safety aspects. *Blood Coagul Fibrinolysis* 1998; **9**: S115–S118.
36 Ehrlich HJ, Henzl MJ, Gomperts ED. Safety of factor VIII inhibitor bypass activity (FEIBA): 10-year compilation of thrombotic adverse events. *Haemophilia* 2002; **8**: 83–90.
37 Abshire T, Kenet G. Safety update on the use of recombinant factor VIIa and the treatment of congenital and acquired deficiency of factor VIII or IX with inhibitors. *Haemophilia* 2008; **14**: 898–902.
38 Sindet-Pedersen S, Stenbjerg S. Effect of local anti-fibrinolytic treatment with tranexamic acid in hemophiliacs undergoing oral surgery. *J Oral Maxillofac Surg* 1986; **44**: 704–7.
39 Ghosh K, Shetty S, Jijina F, Mohanty D. Role of epsilon amino caproic acid in the management of haemophilic patients with inhibitors. *Haemophilia* 2004; **10**: 58–62.
40 Francesconi M, Korninger C, Thaler E, *et al.* Plasmapheresis: Its value in the management of patients with antibodies to factor VIII. *Haemostasis* 1982; **11**: 79–86.
41 Watt RM, Bunitsky K, Faulkner EB, *et al.* Treatment of congenital and acquired hemophilia patients by extracorporeal removal of antibodies to coagulation factors—a review of United States clinical studies. *Transfusion Science* 1992; **13**: 233–53.
42 Freiburghaus C, Berntorp E, Ekman M, *et al.* Immunoadsorption for removal of inhibitors: Update on treatments in Malmö-Lund between 1980 and 1995. *Haemophilia* 1998; **4**: 16–20.
43 Dargaud Y, Lienhart A, Meunier S, *et al.* Major surgery in a severe haemophilia A patient with high titre inhibitor: Use of the thrombin generation test in the therapeutic decision. *Haemophilia* 2005; **11**: 552–8.
44 Teitel JM, Carcao M, Lillicrap D, *et al.* Orthopaedic surgery in haemophilia patients with inhibitors: A practical guide to haemostatic, surgical and rehabilitative care. *Haemophilia* 2009; **15**: 227–39.
45 Teitel J, Berntorp E, Collins P, *et al.* A systematic approach to controlling problem bleeds in patients with severe congenital haemophilia A and high-titre inhibitors. *Haemophilia* 2007; **13**: 256–63.
46 Astermark J, Donfield SM, DiMichele DM, *et al.* A randomized comparison of bypassing agents in hemophilia complicated by an inhibitor: The FEIBA NovoSeven Comparative (FENOC) Study. *Blood* 2007; **109**: 546–51.
47 Schneiderman J, Nugent DJ, Young G. Sequential therapy with activated prothrombin complex concentrate and recombinant factor VIIa in patients with severe haemophilia and inhibitors. *Haemophilia* 2004; **10**: 347–51.

12 Acquired inhibitors to factor VIII

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Epidemiology

Acquired hemophilia (AH) has an estimated prevalence of 1.48 cases per million per year, and a reported mortality between 9% and 22% [1,2]. Despite its low prevalence, the condition imposes significant clinical and medical economic challenges because its dramatic complications are frequently life-threatening and management of bleeding events is expensive.

Age at onset for AH is distributed in a biphasic pattern, with a small peak in young individuals, primarily postpartum women and those with autoimmune diseases, and a major peak in those aged 60–80 years of either sex. AH is uncommon in children younger than age 16 (estimated at 0.045/million/year), and may even be underdiagnosed in the very elderly older than age 85 (estimated at 14.7/million/year) [2]. In the largest published population series, 50–60% of diagnosed individuals were previously healthy with no identified underlying disease state [1–3]. Consistent disease associations have been reported in the other half of the patients and include pregnancy, evolving or pre-existing autoimmune or malignant disorders, and rarely medications [4].

Pathophysiology and characteristics of autoantibodies to factor VIII

Human factor VIII (FVIII) circulates in the plasma, noncovalently bound to von Willebrand factor (VWF) protein, which chaperones it through the circulation. The sequence of the FVIII protein is composed of amino acids grouped into six domains. The most common epitopes for autoantibody (as well as alloantibody) binding appear to lie between amino acids 454–509 and 593 in the A2 domain, between 1804 and 1819 in the A3 domain, and between 2181 and 2243 in the C2 domain [5]. Anti-C2 antibodies inhibit the binding of FVIII to phospholipid and may also interfere with the binding of FVIII to VWF protein, whereas anti-A2 and anti-A3 antibodies impede the binding of FVIII to factor X (FX) and factor IXa (FIXa), respectively, in the intrinsic pathway FX activation complex. Autoantibodies binding to epitope sites other than those mentioned above may be clinically silent, or may be involved in other methods of FVIII inactivation, including mediating FVIII hydrolysis [6]. FVIII antibodies directed against areas in the B domain may influence clearance of FVIII from the circulation.

Most antibodies are mixtures of polyclonal IgG1 and IgG4 immunoglobulins, with the IgG4 molecules mainly responsible for inhibiting clotting activity. Kappa light chains predominate. The IgG4 antibodies do not form immunoprecipitates or fix complement; thus, end-organ damage does not occur as it may with alloantibodies against FIX.

The mechanisms by which FVIII activity is inhibited as a result of interacting with autoantibodies versus alloantibodies are quite dissimilar. Alloantibodies typically neutralize FVIII activity completely, following a so-called linear type I kinetics pattern. In contrast, most autoantibodies do not completely neutralize or inhibit FVIII activity and interact with FVIII via a nonlinear, nonsaturable, complex pattern of type II kinetics. The implications of this latter nonsaturable reaction process are profound: in patients with AH, residual low levels of FVIII activity may be detectable in laboratory assays; however, individuals with AH may bleed as profusely as if they had no FVIII activity [7]. The level of residual FVIII activity in the context of the presence of autoantibody inhibitors does not predict for the severity or frequency of their bleeding complications. Furthermore, these inhibitors, which follow complex kinetics, may only be quantified inexactly since the standard Bethesda assay is based on linear kinetics. This phenomenon results in routine underestimation of the potency of the autoantibody inhibitor. Thus, patients may be classified as having low titers of the autoantibody inhibitor, expressed as Bethesda units (BU), but manifest with severe bleeding [7].

It is apparent also that the contrasting interactions of auto- versus alloantibody inhibitors with FVIII result in very different patterns of clinical bleeding complications. The explanations for this are not obvious; however, in contrast to the hemorrhagic arthropathy, which predominates in individuals with alloantibody inhibitors, AH is associated with more profound visceral, intramuscular, and soft tissue bleeding.

Associated disease states

Auto-FVIII antibody inhibitors frequently are associated with disease states thought to arise from a dysregulated immune

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Table 12.1 Underlying disorders associated with acquired hemophilia.

Associated disease	Green 1981 (n = 178) [1]	Morrison 1993 (n = 65) [8]	Collins 2007 (n = 150) [2]
Idiopathic (%)	46.1	55.4	63.3
Autoimmune (%)	18.0	16.9	16.7
Malignancy (%)	6.7	12.3	14.7
Pregnancy (%)	7.3	10.8	2.0
Drug reaction (%)	5.6	3.1	—
Dermatologic (%)	4.5	1.5	3.3
Other (%)	11.8	—	—

system (see Table 12.1). In the largest series, an autoimmune association was found in ~17% of cases [2,8]. Primary among such are collagen vascular disorders, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Less commonly, AH is associated with more organ-specific autoimmune diseases such as myasthenia gravis, multiple sclerosis, Graves' disease, and autoimmune hemolytic anemia. Associations with asthma, chronic inflammatory bowel disease, and graft versus host disease following allogeneic bone marrow transplant have also been reported anecdotally.

Development of an autoanti-FVIII inhibitor is a rare but well-recognized complication of pregnancy. The prognosis is overall good and mortality rates are low (0–6%) [4]. The most common manifestation is postpartum bleeding in the 1–4 months after delivery [9]. The development of AH has been described up to a year post partum, but these cases may be more likely associated with evolving or occult autoimmune disorders [10]. Auto-FVIII inhibitors may actually develop in 2–14% of otherwise normal pregnancies but remain clinically insignificant. Multiparous women may have low levels of such antibodies detectable in their plasmas for many years. The pathogenesis of these pregnancy-related autoantibody inhibitors directed against FVIII remains unclear.

Theoretically and anecdotally, the anti-FVIII IgG autoantibodies circulating in pregnant mothers with clinical AH can increase the risk of severe hemorrhagic complications in the neonate at delivery owing to the transplacental transfer of the antibody. Because these bleeds often involve the central nervous system, cesarean section delivery should be seriously considered in these patients. If the inhibitor is low titer (≤5 BU), it generally disappears spontaneously over a median of 30 months post partum and may rarely (<15%) recur with subsequent pregnancies. High-titer inhibitors (>5 BU) can persist for years despite treatment with corticosteroids, intravenous immunoglobulin, and cytotoxic agents, and may precede the development of an overt autoimmune disorder such as SLE or RA.

Associated malignancies, most commonly lymphoproliferative in nature, have been identified in up to 15% of patients with AH, and may be associated with a worse prognosis [4,11]. The association with cancer occurs predominantly in elderly men and, especially when observed in conjunction with lymphoproliferative disorders, is consistent with the broad range of autoimmune phenomena that frequently complicate these conditions. The etiologic role of solid tumor malignancies in AH is not so apparent. In fact, some authors consider that the appearance of FVIII autoantibodies in patients with solid tumors such as prostate cancer may well be an epiphenomenon as these neoplasms occur so commonly in the same elderly cohort as AH. In some cases, the FVIII autoantibodies have been observed to arise after treatment for cancer has been initiated; it is possible that use of corticosteroids, cytotoxic agents, and biologic response modifiers, such as interferon-α, could have altered host immunity and predisposed the patient to the development of autoimmune phenomena. Alternatively, FVIII autoantibody inhibitors could represent a host immune response to the tumor-derived antigens, although no tumor antigen has yet been described to have homology to FVIII. The presence of an underlying malignancy is not a contraindication to the use of immunotherapy to suppress the production of the antibody even in cases that fail to respond to primary antitumor therapy [12]. The auto-FVIII antibody inhibitor may not remit following successful eradication of the malignancy. Conversely, the re-emergence of inhibitors is not a reliable indication of tumor recurrence in patients.

Drug reactions to certain medications, including antibiotics such as pencillin and its derivatives, sulfonamides, and chloramphenicol; anticonvulsants such as diphenylhydantoin; and BCG (bacille Calmette–Guérin) vaccination have all been associated with the development of antibodies to factor VIII [1,13]. Frequently, drug-induced anti-FVIII antibodies arise after hypersensitivity reactions and remit shortly after withdrawing the offending drug. The pathophysiology remains unknown in most cases; however, the significant alterations of immune function which are induced by the administration of such medications as interferon-α and fludarabine may facilitate the appearance of autoantibodies against FVIII, as they do for other immune phenomena reported with their use, e.g., immune thrombocytopenic purpura or autoimmune hemolytic anemia.

Clinical manifestations of acquired hemophilia

The clinical picture of AH is characterized by acute onset of severe bleeding in individuals who previously had no history of bleeding diatheses. It is notable that the bleeding pattern is distinctly more severe and anatomically varied than that observed in congenital severe hemophilia A complicated by alloimmune inhibitors against FVIII. Patients generally present with mucocutaneous bleeding such as epistaxis and gastrointestinal bleeding, as well as soft tissue bleeding including extensive ecchymoses and hematomas. Joint and muscle bleeding, commonly experienced by patients with congenital hemophilia A and alloinhibitors, is comparatively rare. The bleeding

is usually spontaneous, although minimal trauma or surgical procedures may predispose to disproportionately extensive ecchymoses and bleeding. Patients may present with overt bleeding or anemia because of occult hemorrhage. The bleeding is accompanied by considerable morbidity; however, mortality, ranging between 9% and 22% [1,2] frequently is a result of the infectious complications of immunosuppression rather than fatal hemorrhage.

Laboratory diagnosis

The activated partial thromboplastin time (aPTT) is prolonged when FVIII (or FIX, FXI, and FXII), for which it screens, is decreased in undiluted patient plasma. The prothrombin time (PT) and platelet function are usually normal. To determine if the elevated aPTT is a result of a specific clotting factor deficiency or a pathologic circulating anticoagulant, performance of mixing studies is critical. For FVIII inhibitory antibodies of the allo- or auto-variety, the neutralizing expression of the inhibitor is time- and temperature-dependent and may require 2 h at 37°C, especially in the case of weak autoantibodies, before an accurate assessment of the inhibitor can be ascertained. A difference of 10 s or greater is indicative of a positive inhibitor screen.

To confirm the neutralization of a specific coagulation factor by auto- or allo-antibody inhibitors, assays for each of the coagulation factors in the involved pathway must be performed. Lupus-like anticoagulants can be distinguished from clotting factor autoantibody inhibitors by the finding of a positive platelet neutralization assay and/or a prolonged dilute Russell's viper venom test (dRVVT), tissue thromboplastin inhibition assay, or kaolin clotting time. In addition, clinical presentation is essential to distinguishing among these possibilities, as lupus-like anticoagulants are typically found in either asymptomatic or hypercoagulable patients, and patients with AH tend to bleed.

When the presence of a specific factor inhibitor is suspected, it is imperative that the target is identified and the degree of inhibitory activity quantified. This is accomplished by incubating a source of the specific clotting factor (typically, pooled normal plasma) with increasing dilutions of the patient's plasma at 37°C for 2 h. As the antibody-containing plasma is diluted, the clotting factor concentration will appear to increase, although the baseline mixture may yield a normal aPTT or FVIII activity level. The inhibitor potency is expressed most commonly worldwide in terms of BU, where 1.0 BU is the reciprocal dilution of patient test plasma permitting detection of 50% residual FVIII activity in a mixture with normal pooled plasma [14]. The Nijmegen modification of the Bethesda assay employs buffered normal plasma throughout each step of the assay, thereby minimizing shifts in pH and allowing for increased sensitivity of the inhibitor assay to detect low-titer inhibitors, e.g., ≤0.6 BU [15]. While this assay is widely accepted for research trials, particularly those in which the detection of low-titer alloantibody inhibitors is critical, it is labor intensive and is rarely needed or used in the typical clinical scenario for AH.

Other assays for detecting specific inhibitors which target clotting factor proteins rely on immunologic rather than functional methodologies. These assays, such as enzyme-linked immunoabsorbent assays (ELISAs), are extremely sensitive and may detect antibodies that do not inhibit FVIII activity in vitro or in vivo. They have thus far generally been confined to a research setting.

Treatment

There are two major goals for the treatment of AH: the immediate control of acute and chronic bleeding and the long-term suppression/eradication of the autoantibody inhibitor. The first objective is necessary because bleeding episodes are often relentless without reversal of the coagulation deficit, and can be life threatening. The second objective is required to restore normal hemostasis and can usually be accomplished using some type of immunotherapy. An important caveat is that the level of inhibitor potency is not directly proportional to or predictive of the severity or frequency of bleeding events.

The choice of therapeutic agent to reverse bleeding depends on the severity of the bleeding, the clinical setting, and the initial and historical peak titers of antihuman FVIII inhibitors (Figure 12.1). Several strategies, such as administration of desmopressin and concentrates of human recombinant FVIII, may raise the FVIII activity levels adequately in plasma of individuals with low-titer auto-FVIII antibody inhibitors (≤5 BU). If the inhibitor titer is high (>5 BU), or if bleeding persists despite infusions of FVIII concentrates, then FVIII bypassing agents, such as aPCCs or recombinant factor VIIa (rFVIIa), are indicated. Local measures for treatment of mucosal hemorrhage, such as antifibrinolytic agents or topical fibrin glues, may also be helpful. The use of plasma-derived or recombinant topical thrombin preparations may also spare the need for more expensive parenteral replacement products.

The target level of FVIII activity needed to control most bleeding events is 30–50% of normal. This is generally more feasible to achieve if the inhibitor titer is <5 BU. The recommended initial dose of FVIII concentrate is 20 IU/kg for each BU of inhibitor plus 40 additional IU/kg intravenously as a bolus. The plasma FVIII activity level should be determined 10–15 min after the initial bolus and, if the incremental recovery is not adequate, another bolus dose should be administered [16]. An alternative approach is to administer an initial intravenous bolus of 200–300 IU/kg followed by continuous infusion of about 4–14 IU/kg/h [17]. These doses are estimations, and FVIII levels and clinical response should be monitored carefully throughout treatment. FVIII concentrates are generally not useful if the patient has a high-titer (>5 BU) or high-responding inhibitor. The latter may not be known until

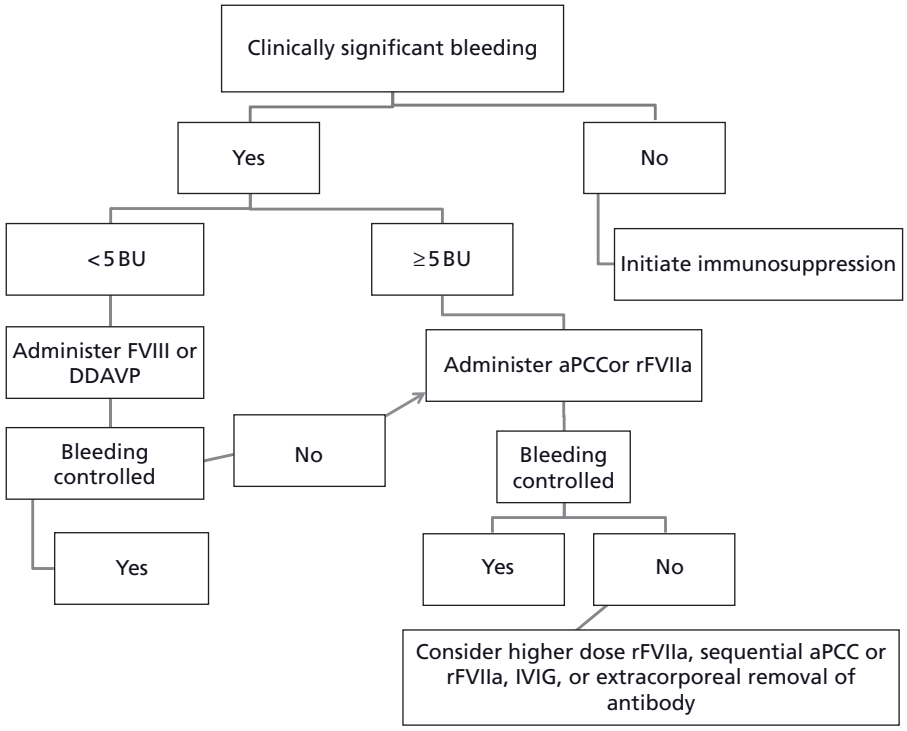

Figure 12.1 Treatment algorithm 1: Management of acute bleeds.

after the first dose of FVIII is administered as patients with low-titer inhibitors may be high responders, with the capacity to mount an anamnestic response after exposure to the offending antigen, e.g., FVIII.

The FVIII present in intermediate-purity concentrates is associated with a large amount of VWF protein, which theoretically protects the FVIII from proteolytic inactivation and neutralization by the autoantibodies in the patient's plasma, particularly by antibodies directed against C2 domain. Thus far, this has been more of an in vitro than an in vivo consideration, but it is provocative and awaits testing in a clinical setting [7,18]. A similar argument has been posited for the benefits of 1-deamino-8-D-arginine vasopressin (DDAVP) for the treatment of autoantibody FVIII inhibitors, albeit low-titer ones. In this situation, DDAVP would be expected to increase both VWF protein and FVIII release into the plasma.

Porcine plasma-derived FVIII (pFVIII) concentrate was formerly considered a vital first-line therapy to achieve hemostasis in patients with AH because of both its excellent efficacy and the opportunity for close laboratory monitoring (unlike the bypassing agents, aPCCs and rFVIIa, postinfusion FVIII activity levels may be followed in the laboratory while using pFVIII). Unfortunately, pFVIII was removed from the market in 2004 because of its contamination with porcine parvovirus. In contrast to allo-FVIII antibody inhibitors, the crossreactivity between antihuman FVIII autoantibodies and pFVIII is usually minimal and rarely neutralizing enough to obviate the use of pFVIII concentrate [3]. A new recombinant porcine B-domain deleted FVIII product (OBI-1, Ipsen) has recently completed phase I and II clinical trials in congenital hemophilia and allo-inhibitors [19]; trials in acquired hemophilia are in the process of being designed and look promising for the future.

For patients with high-titer inhibitors (>5 BU), the "bypassing" agents, aPCCs and rFVIIa, are the most commonly used first-line therapies. Factor Eight Inhibitor Bypassing Activity (FEIBA®, Baxter) is currently the only commercially available aPCC in the USA. Its main active components include activated FX and prothrombin, but the precise mechanism for its hemostatic action in AH remains poorly understood. aPCCs have been used extensively in the treatment of bleeding episodes in patients with both allo- and auto-FVIII antibody inhibitors. Retrospective studies in subjects with AH report excellent or good hemostatic response in 86–100% of cases [20,21]. For treatment of acute bleeds, the recommended dose of FEIBA is 50–100 IU/kg infused intravenously every 8–12 h. Minor reactions such as headache, nausea, pruritus, skin rashes, and diarrhea have been noted. Rare cases of disseminated intravascular coagulation (DIC), venous thromboembolism, and myocardial infarction have also been reported [22]. There is also a small, but theoretical, risk of viral and perhaps prion transmission as it is a plasma-derived product. This necessitates a careful risk to benefit analysis prior to the administration of this human plasma-derived product. It must be noted that there has never been a reported case of human immunodeficiency virus (HIV), hepatitis C virus (HCV), or variant Creutzfeldt–Jakob disease transmission associated with the use of FEIBA.

rFVIIa (NovoNordisk) is the other widely used bypassing agent. While its mechanism of action is also not fully understood, it has been demonstrated to directly activate FX and increase thrombin production on the surface of activated platelets even in the absence of factors VIII and IX. The activated platelet specific generation of thrombin is postulated to localize its action to sites of active bleeding and tissue injury. Retrospective data collected from compassionate use programs [23] and the Hemophilia and Thrombosis Research Society (HTRS) database suggest that rFVIIa is effective or partially effective in 88% of evaluable bleeding episodes. When used first line, 95% efficacy was observed, and 83% efficacy when used as salvage treatment [24]. Rapid intravenous bolus administration of 90–120 μg/kg rFVIIa, repeated every 2–3 h, depending on clinical response, is recommended. Continuous infusion of rFVIIa is being explored as a means of simplifying the demands of frequent dosing and of reducing cost of product. The major adverse effect associated with rFVIIa, as with FEIBA, is the occurrence of arterial and venous thrombosis, which generally occurs at very low rates in AH, and has been noted predominantly in individuals with pre-existing atherosclerotic disease [25,26]. The frequent dosing interval and higher baseline cost can make administration of rFVIIa prohibitively expensive for patients in developing countries.

Although no randomized trials have been performed comparing FEIBA with rFVIIa in patients with AH, unblinded randomized trials in subjects with congenital hemophilia and alloinhibitors being treated for joint bleeding suggest that FEIBA and rFVIIa appear to exhibit a similar effect on joint bleeds, that individual patients frequently respond differently to the two products, and that using a higher dose of rFVIIa (270 μg/kg) (in children and young adults) may reduce the frequency and number of infusions required to treat a bleed [27,28]. If first-line therapy fails, switching to the alternative bypassing agent may prove successful. As patients with AH are older and more likely to have pre-existing risk factors for arterial and venous thrombosis, escalating or sequential use of either bypassing agent should proceed with great caution [29,30]. Secondary prophylaxis to diminish bleeding has been used in patients with congenital hemophilia and inhibitors, but has not yet been explored in AH. An important drawback to the use of both products is the lack of a validated laboratory technique to monitor or predict hemostatic efficacy and safety. The use of thrombin generation assays and thromboelastography is currently being explored in research settings [31]. Currently, when administering bypassing agents in AH, efficacy is the clinical endpoint and dosing is empirical.

The primary aim in long-term management of AH is to eradicate the FVIII autoantibodies so that further bleeding can be averted. Although in some clinical situations (postpartum women and drug-related AH) FVIII autoantibodies may remit spontaneously, most published guidelines and algorithms recommend early initiation of eradication therapy. This is usually achieved through immunosuppressive medications or immunomodulation.

Successful immunosuppression regimens in AH have most frequently used corticosteroids as the cornerstone, either as a single agent or in combination with cyclophosphamide (Figure 12.2). In a prospective randomized trial, Green *et al.* treated

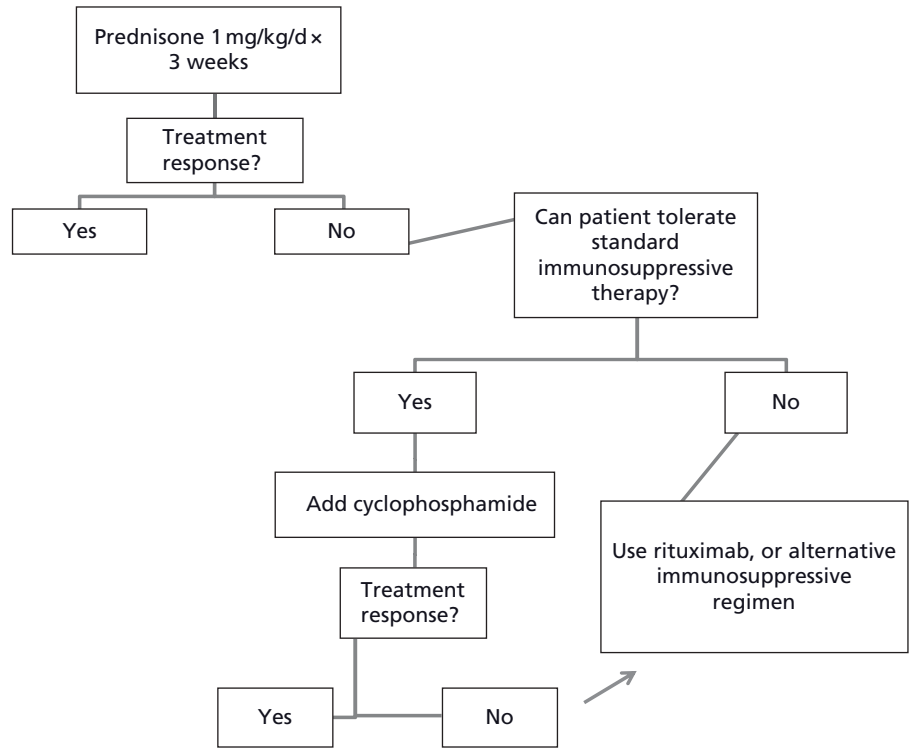

Figure 12.2 Treatment algorithm 2: Immunosuppression.

31 subjects with prednisone 1 mg/kg/day for 3 weeks, with a 32% complete remission (CR) rate. After 3 weeks, in subjects with persistence of antibody, switching to oral cyclophosphamide 2 mg/kg/day as second-line therapy appeared more effective than continuing prednisone alone (CR rate 50% vs. 42%) [32]. While some investigators have advocated using combination therapy first line, a meta-analysis of 20 reports and a UK surveillance study showed that although addition of cyclophosphamide improved initial CR rates, there was no change in overall survival [33]. This may be related to the high rates of adverse effects from immunosuppressive agents, including neutropenia-related infections, in this predominantly elderly population [4]. Patients who respond to steroids fall into a good prognostic category. Median time to remission is 5–7 weeks, with overall CR rates ranging between 60% and 80% [34].

Other immunosuppressive medications have been employed for particularly refractory autoantibody inhibitor eradication, including azathioprine, cyclosporine, FK506 (tacrolimus), mycophenolate mofetil (Cellcept®), sirolimus (Rapamycin®), and 2-chlorodeoxyadensine [35]. Controlled studies have not been performed to confirm their comparative safety and efficacy in sufficiently large populations.

Introduction of rituximab, a chimeric monoclonal antibody that targets the CD20 antigen, has opened new horizons in the treatment of benign hematologic diseases, and has been used to treat inhibitors in patients with both congenital and acquired hemophilia. Anecdotal case reports and series including over 65 patients have reported CR rates >80%, which must be interpreted with some caution because of the likely bias of publishing only positive results. While this justifies a randomized prospective trial to define the exact role of rituximab in the management of AH, it cannot currently be recommended as first-line therapy [36,37]. Some such small trials are under way.

Other less frequently used treatment options include administration of intravenous immunoglobulin in large doses, which often mediates a rapid but short-lived decline in autoantibody titers, perhaps owing to anti-idiotypic antibodies derived from the pooled plasmas of thousands of normal donors. The usual administered dose is 2 g/kg divided in either two or five daily infusions. Intravenous immunoglobulin is rarely able to induce a CR when used alone (25–37% response rates, effective probably only in low-titer inhibitors) [38], but may be useful adjunctive therapy along with immunosuppressants, as part of an immune tolerance induction (ITI) regimen, or with extracorporeal plasmapheresis [39]. Combination treatment with FVIII concentrate, cyclophosphamide, and methylprednisolone, or other similar immunomodulation protocols, has reported high success rates (CR 93%) of inhibitor eradication with low recurrence rates [40]. These excellent results remain to be tested in a randomized manner but appear to be a promising, albeit costly, treatment strategy.

In patients with AH, relapse rates of up to 20% have been reported, at a median of 7.5 months (range 1 week to 14 months) [2]. In over 50% of patients, a second CR could be induced, although some patients required longer term immunosuppression. Patients should be carefully followed after remission and advised to promptly report new hemorrhagic symptoms so that relapses may be detected and treated as rapidly as possible.

Additional new treatment modalities for AH await development and clinical research application. Theoretically, they could include the manufacture via recombinant technology of a preparation of combined rFII–rFX, which could provide an alternative to rFVIIa concentrate for refractory auto-FVIII inhibitors; the development of recombinant pFVIII concentrate (currently in phase III clinical trials) and the development of a human–porcine FVIII hybrid molecule, with porcine-derived amino acid substitutions in the A2, A3, and C2 domains of the human FVIII molecule, areas which serve as the epitopes for autohuman FVIII antibody inhibitors; and the development of new rFVIIa constructs which have more potent dose–response generation of "thrombin bursts." Finally, modulation of the immune system may be possible to eradicate the autoantibody. Anti-CD40 ligand monoclonal antibodies were used with some success with allo-FVIII antibodies before being withdrawn from the clinical arena because of hypercoagulability adverse events in an RA population. This approach remains to be tested for auto-FVIII antibodies. All of these strategies are provocative and suggest that the future for treatment of the auto-FVIII antibody inhibitors in acquired hemophilia is promising.

References

1 Green D, Lechner K. A survey of 215 non-hemophilic patients with inhibitors to Factor VIII. *Thromb Haemost* 1981; **45**: 200–3.

2 Collins PW, Hirsch S, Baglin TP, *et al.* Acquired hemophilia A in the United Kingdom: A 2-year national surveillance study by the United Kingdom Haemophilia Centre Doctors' Organisation. *Blood* 2007; **109**: 1870–7.

3 Kessler CM, Ludlam CA. The treatment of acquired factor VIII inhibitors: Worldwide experience with porcine factor VIII concentrate. International Acquired Hemophilia Study Group. *Semin Hematol* 1993; **30**(Suppl. 1): 22–7.

4 Delgado J, Jimenez-Yuste V, Hernandez-Navarro F, Villar A. Acquired haemophilia: Review and meta-analysis focused on therapy and prognostic factors. *Br J Haematol* 2003; **121**: 21–35.

5 Lollar P. Pathogenic antibodies to coagulation factors. Part one: Factor VIII and factor IX. *J Thromb Haemost* 2004; **2**: 1082–95.

6 Lacroix-Desmazes S, Moreau A, *et al.* Catalytic activity of antibodies against factor VIII in patients with hemophilia A. *Nat Med* 1999; **5**: 1044–7.

7 Boggio LN, Green D. Acquired hemophilia. *Rev Clin Exp Hematol* 2001; **5**: 389–404.

8 Morrison AE, Ludlam CA, Kessler C. Use of porcine factor VIII in the treatment of patients with acquired hemophilia. *Blood* 1993; **81**: 1513–20.

9 Michiels JJ. Acquired hemophilia A in women postpartum: Clinical manifestations, diagnosis, and treatment. *Clin Appl Thromb Hemost* 2000; **6**: 82–6.

10 Solymoss S. Postpartum acquired factor VIII inhibitors: Results of a survey. *Am J Hematol* 1998; **59**: 1–4.

11 Hauser I, Lechner K. Solid tumors and factor VIII antibodies. *Thromb Haemost* 1999; **82**: 1005–7.

12 Sallah S, Wan JY. Inhibitors against factor VIII in patients with cancer. Analysis of 41 patients. *Cancer* 2001; **91**: 1067–74.

13 Franchini M, Capra F, Nicolini N, *et al.* Drug-induced anti-factor VIII antibodies: A systematic review. *Med Sci Monit* 2007; **13**: RA55–61.

14 Kasper CK, Aledort L, Aronson D, *et al.* Proceedings: A more uniform measurement of factor VIII inhibitors. *Thromb Diath Haemorrh* 1975; **34**: 612.

15 Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: Improved specificity and reliability. *Thromb Haemost* 1995; **73**: 247–51.

16 Kasper CK. Human factor VIII for bleeding in patients with inhibitors. *Vox Sang* 1999; **77**(Suppl. 1): 47–8.

17 Blatt PM, White GC, 2nd, McMillan CW, Roberts HR. Treatment of anti-factor VIII antibodies. *Thromb Haemost* 1977; **38**: 514–23.

18 Berntorp E. Variation in factor VIII inhibitor reactivity with different commercial factor VIII preparations: Is it of clinical importance? *Haematologica* 2003; **88**: EREP03.

19 Mahlangu J, Andreeva TA, Macfarlane D, *et al.* A phase II open-label study evaluating hemostatic activity, pharmacokinetics and safety of recombinant porcine factor VIII (OBI-1) in hemophilia A patients with alloantibody inhibitors directed against human FVIII. *Blood* 2007; **110**: 783.

20 Sallah S. Treatment of acquired haemophilia with factor eight inhibitor bypassing activity. *Haemophilia* 2004; **10**: 169–73.

21 Holme PA, Brosstad F, Tjonnfjord GE. Acquired haemophilia: Management of bleeds and immune therapy to eradicate autoantibodies. *Haemophilia* 2005; **11**: 510–15.

22 Mehta R, Parameswaran R, Shapiro AD. An overview of the history, clinical practice concerns, comparative studies and strategies to optimize therapy of bypassing agents. *Haemophilia* 2006; **12**(Suppl. 6): 54–61.

23 Hay CR, Negrier C, Ludlam CA. The treatment of bleeding in acquired haemophilia with recombinant factor VIIa: A multicentre study. *Thromb Haemost* 1997; **78**: 1463–7.

24 Sumner MJ, Geldziler BD, Pedersen M, Seremetis S. Treatment of acquired haemophilia with recombinant activated FVII: A critical appraisal. *Haemophilia* 2007; **13**: 451–61.

25 O'Connell KA, Wood JJ, Wise RP, Lozier JN, Braun MM. Thromboembolic adverse events after use of recombinant human coagulation factor VIIa. *JAMA* 2006; **295**: 293–8.

26 Abshire T, Kenet G. Safety update on the use of recombinant factor VIIa and the treatment of congenital and acquired deficiency of factor VIII or IX with inhibitors. *Haemophilia* 2008; **14**: 898–902.

27 Astermark J, Donfield SM, DiMichele DM, *et al.* A randomized comparison of bypassing agents in hemophilia complicated by an inhibitor: The FEIBA NovoSeven Comparative (FENOC) Study. *Blood* 2007; **109**: 546–51.

28 Young G, Shafer FE, Rojas P, Seremetis S. Single 270 microg kg(−1)-dose rFVIIa vs. standard 90 microg kg(−1)-dose rFVIIa and APCC for home treatment of joint bleeds in haemophilia patients with inhibitors: A randomized comparison. *Haemophilia* 2008; **14**: 287–94.

29 Miranda GG, Rodgers GM. Treatment of an acquired factor VIII inhibitor with sequential recombinant factor VIIa and FEIBA. *Haemophilia* 2009; **15**: 383–5.

30 Sood S, Konkle BA, Kessler CM, Cooper DL, HRS-HTRS Registry Investigators. Treatment of Acute Bleeds in Acquired Hemophilia: Analysis from the Hemophilia Research Society (HRS) and Hemophilia and Thrombosis Research Society (HTRS) Registry. *ASH Annual Meeting Abstracts* 2008; **112**: 2285.

31 Barrowcliffe TW. Monitoring inhibitor patients with the right assays. *Semin Hematol* 2008; **45**(Suppl. 1): S25–30.

32 Green D, Rademaker AW, Briet E. A prospective, randomized trial of prednisone and cyclophosphamide in the treatment of patients with factor VIII autoantibodies. *Thromb Haemost* 1993; **70**: 753–7.

33 Hay CR, Brown S, Collins PW, Keeling DM, Liesner R. The diagnosis and management of factor VIII and IX inhibitors: A guideline from the United Kingdom Haemophilia Centre Doctors Organisation. *Br J Haematol* 2006; **133**: 591–605.

34 Collins PW. Treatment of acquired hemophilia A. *J Thromb Haemost* 2007; **5**: 893–900.

35 Ma AD, Carrizosa D. Acquired factor VIII inhibitors: Pathophysiology and treatment. *Hematology Am Soc Hematol Educ Program* 2006: 432–7.

36 Stasi R, Brunetti M, Stipa E, Amadori S. Selective B-cell depletion with rituximab for the treatment of patients with acquired hemophilia. *Blood* 2004; **103**: 4424–8.

37 Franchini M. Rituximab in the treatment of adult acquired hemophilia A: A systematic review. *Crit Rev Oncol Hematol* 2007; **63**: 47–52.

38 Schwartz RS, Gabriel DA, Aledort LM, Green D, Kessler CM. A prospective study of treatment of acquired (autoimmune) factor VIII inhibitors with high-dose intravenous gammaglobulin. *Blood* 1995; **86**: 797–804.

39 Zeitler H, Ulrich-Merzenich G, Hess L, *et al.* Treatment of acquired hemophilia by the Bonn–Malmo Protocol: Documentation of an *in vivo* immunomodulating concept. *Blood* 2005; **105**: 2287–93.

40 Nemes L, Pitlik E. New protocol for immune tolerance induction in acquired hemophilia. *Haematologica* 2000; **85**(Suppl.): 64–8.

13 Hemophilia B—molecular basis

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Introduction

Classic hemophilia B (HB) is caused by mutations in the *F9* gene that lead to quantitative and/or qualitative deficiencies in circulating factor IX (FIX) protein. Genetic analysis is now a standard part of the diagnostic process in many hemophilia centers and has resulted in a much greater understanding of the etiology of this disorder. This facilitates screening of relatives and in the future may influence therapeutic options for individual patients. From a scientific perspective it has led to a greater appreciation of the relationship between the structure of the FIX protein and its function. This is of fundamental importance for research and has been vital for breakthroughs such as the production of recombinant FIX. It is a cornerstone for current and future research streams such as the development of better recombinant proteins or effective gene therapy.

The process of understanding the molecular basis of HB began with characterization of *F9* in the early 1980s. Several groups described parts of the *F9* coding sequence between 1982 and 1984, and the full sequence of the entire gene was published in 1985 [1]. *F9* is located at Xq27.1 and spans 33 kbp. There are eight exons which are transcribed into a 2802-bp mRNA. This, in turn, is translated into a 461-amino acid (aa) polypeptide, from which removal of a 28-aa signal peptide and 18-aa propeptide leaves a 415-aa mature protein. The primary translation product has an estimated molecular mass of 52 kDa. The two principal activators of this zymogen are FXIa and the TF.FVIIa complex. Activation occurs following a double cleavage after Arg192 and Arg226. This removes the 35-aa activation peptide which lies between the EGF2 (epidermal growth factor-like) and SP (serine protease or catalytic) domains. The active enzyme is a two-chain serine protease with a light chain of 145 aa containing the Gla, EGF1, and EGF2 domains and a heavy chain of 235 aa containing the SP domain. The neo N-terminus of the heavy chain is inserted into the catalytic domain during activation and the two chains are held together by the Cys178–Cys335 disulfide bond. The relationship between the nucleotide sequence and the translated polypeptide is shown in Figure 13.1.

The Gla domain contains 11 glutamic acid residues that are post-translationally modified to 4-carboxyglutamate. These are essential for binding the calcium ions that give the Gla domain the positive charge that is required for efficient interaction with a negatively charged phospholipid membrane. The catalytic triad, characteristic of all members of the serine protease family to which FIX belongs, is made up of His267, Asp315, and Ser411. On its own, FIXa is a relatively inefficient enzyme in the activation of its preferred substrate, FX. This activity is enhanced when the enzyme is noncovalently bound to its cofactor, FVIIIa, to form the macromolecular *intrinsic tenase* complex. The maximum catalytic activity of this complex is achieved after docking on a negatively charged phospholipid membrane. The effect of FVIIIa is to increase the specific activity by some 50 000-fold over that of the enzyme in isolation [2].

The structure of the macromolecular intrinsic tenase complex has yet to be elucidated but can be inferred from the published crystal structures of porcine FIXa [3], individual domains of human FIXa [4–6] and human FVIII [7,8]. In the active enzyme the light chain forms a stalk with the N-terminal Gla domain, anchoring it to the phospholipid membrane. The catalytic domain is a globular structure sitting at the top of the stalk (Figure 13.2). The interface with FVIIIa is formed by one side of the protein and includes critical groups of residues in the EGF1, EGF2, and catalytic domains [8,9].

Techniques for mutation detection

Early methods of mutation detection generally relied upon distinguishing abnormal alleles by virtue of change in size of DNA fragments generated by restriction endonucleases. This is the basis of methods such as Southern blot, which can readily detect large deletions but are generally not suitable for direct detection of missense mutations. As discussed below, most cases of HB are associated with missense mutations. In this situation the disease-associated allele could sometimes be tracked in a pedigree by linkage with known polymorphisms that modified restriction sites [restriction fragment length polymorphisms (RFLPs)]. There are several well-described polymorphisms that can be used for this purpose [10]. One of these is a single nucleotide polymorphism (SNP), G/A g.20393, which codes for alanine or threonine at position 194. This has been termed the Malmö polymorphism and although it has no effect on protein function, it does alter the epitope profile which produces variability in the results of antigen assays

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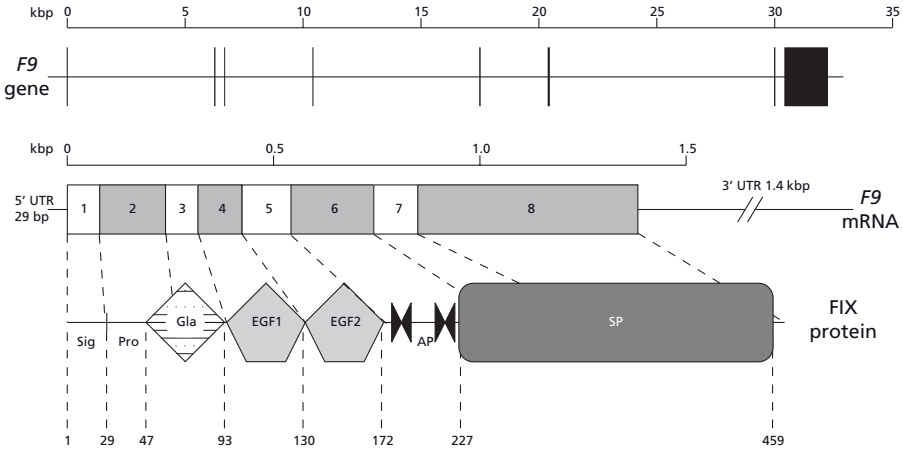

Figure 13.1 Relationship between the *F9* gene and the translated factor IX polypeptide. Exons are depicted as vertical bars in *F9* with thickness approximating to size. In the primary mRNA the parts of the coding sequence transcribed from each exon are shown by numbered blocks. The regions of the protein encoded by different exons are shown by dashed lines and below the protein residue numbering indicates the domain boundaries. The signal and propeptides are removed to form the mature protein. AP, activation peptide; EGF, epidermal growth factor; Pro, propeptide; Sig, signal peptide; SP, serine protease.

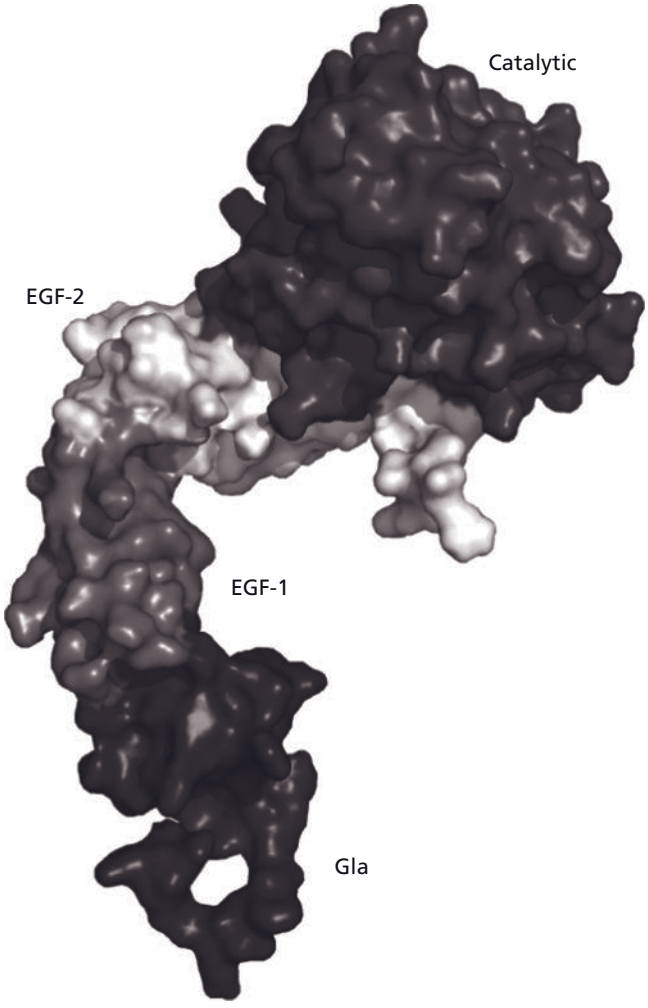

Figure 13.2 Crystal structure of porcine activated factor IX showing the domains in various shades of grey. PDB ID: 1PFX. (See also Plate 13.2.)

from different kits. The importance of these polymorphisms is that they can be used as indirect markers of mutation if the sequence at these positions in a pedigree varies between the normal and abnormal allele. Indirect methods require carriers to be heterozygous at the polymorphic marker, and there is always the possibility that recombination might lead to loss of the linkage with the mutated residue across generations. Therefore, these techniques have been superseded by DNA amplification using polymerase chain reaction (PCR) followed by direct sequencing. The primers and reaction conditions for amplification of the *F9* gene are now well standardized, making this a rapid and robust technique. The amplicons cover the coding sequence and known regulatory regions of *F9*. As PCR of the gene requires a number of amplification reactions the possibility of reducing this by using mRNA as the target has been explored. Unfortunately, aberrant *F9* transcripts are found in peripheral blood, so this approach has largely been abandoned as a routine screening technique [11].

PCR is generally not able to detect large deletions or other gross abnormalities in carriers because of the presence of the other (normal) allele. Although gross abnormalities represent a small group they are nearly always associated with severe disease, making the detection of carrier status important. In the last few years the development of new techniques has helped to tackle this problem. Of these, multiplex ligation-dependent probe amplification (MLPA) and multiplex amplification and probe hybridization (MAPH) seem to be the most promising [12]. In both techniques probes to specific sequences within the gene are amplified. The products from different probes vary by size and are separated and quantified by electrophoresis. As the amount of amplified product is proportional to the amount of starting template, a deleted region will produce a lower peak from the corresponding probe. Already there are reports of the successful application of these techniques to *F9* and it is likely that they will become increasingly used in the near future [13].

Mechanisms of mutation in the *F9* gene

One of the purposes of genetic analysis is to enable phenotypic predictions based on experience of similar mutations in other patients. This is facilitated by the collection of mutation and

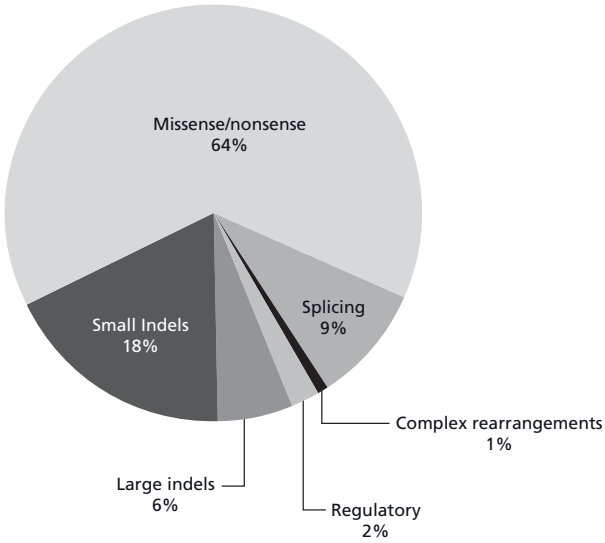

Figure 13.3 Pie chart showing the different types of genetic defects reported in hemophilia B. The percentages are derived from the three public databases listed at the end of the chapter.

phenotype information in databases. Details of three of the largest publicly available databases for *F9* mutations are given at the end of this chapter [14–16]. Across the three databases there are some 900 distinct mutations. The different types of genetic defects that are found in HB are summarized in Figure 13.3.

Single nucleotide changes

Mutations in which a single nucleotide is replaced by another nucleotide (point mutations) are by far the most common type of abnormality found in HB (64% of all mutations). Generally, they are caused by DNA polymerases adding the wrong nucleotide during replication. Different mammalian DNA polymerases vary in their fidelity but the average error rate is in the region of 1 in 5000–10 000 nucleotides. This would lead to a disastrously high rate of mutations without repair mechanisms such as the 3′ to 5′ proofreading activity of DNA exonucleases. These enzymes may be part of the same macromolecular complex as the polymerase and their purpose is to remove an incorrectly incorporated nucleotide. When these repair mechanisms fail a mutation occurs. Analysis of the number of mutations reported at each position demonstrates that certain nucleotides, or groups of nucleotides, are more frequently mutated than others. The occurrence of these so-called "mutation hotspots" indicates that sequence can influence the error rate. An example of this is the over-representation of arginine residues in the databases. This is because of the well-described effect of CpG islands. The nucleotide code for arginine is CGn, which is the target sequence for DNA methyltransferases. These enzymes convert cytosine to 5-methylcytosine, which can be deaminated to form thymine. Thus, CGn is replaced with TGn, which codes for cysteine, tryptophan, or termination. Although deamination can occur to unmethylated cytosine as well, this results in a conversion to uracil, which is not a viable nucleotide in DNA and therefore corrected.

When point mutations causing HB occur spontaneously during meiosis they do so more frequently in the male gamete. Population studies indicate that when a sporadic mutation results in a carrier female it is approximately eight times more likely to have originated in the paternal gamete [17,18]. A similar male-to-female bias has been reported in other X-linked recessive disorders. The reason for this bias is not clear. A higher rate of CpG transitions in the male has been largely discounted. It may be that the baseline rate of mutation is similar in both gametes but that the presence of a normal DNA strand in the female allows repair mechanisms dependent on homologous recombination. Another factor may be the much greater number of meiotic cycles involved in spermatogenesis compared with oogenesis.

The effects of point mutations vary greatly depending on where they occur in the gene. Alterations in the coding sequence can lead to missense mutations, premature stop codons, or splicing defects. Missense mutations can provide a great deal of information about the function of specific parts of the protein. In order to classify mutations they are grouped into two broad types. Type I or quantitative mutations result in reduced levels of an otherwise normal protein. Type II or qualitative mutants have reduced function and may be present in normal or reduced amounts. The separation of mutants into these two classes requires measurement of both protein function (activity or FIX:C) and antigen (FIX:Ag), but unfortunately many laboratories do not routinely measure FIX:Ag. The terms cross-reacting material (CRM) negative and CRM positive (or CRM reduced) approximate to types I and II.

Mutations affecting the signal peptide (residues 1–28) produce the full spectrum of disease severity but with similar levels of antigen and activity. This part of the polypeptide directs intracellular trafficking of the protein and defects can lead to reduced levels of secretion. As the signal peptide is cleaved prior to secretion it plays no part in the function of the circulating form and thus mutations are type I. In contrast, mutation of any of the triad of residues forming the active site invariably causes severe HB because the protein is then incapable of catalytic activity. FIX:C is therefore always <1 IU/dL. However, the amount of protein in the circulation, as measured by the FIX:Ag, is variable and quite often normal as seen with His267Arg, His267Gln, Ser411Gly, and Ser411Arg. If antigen is normal this is an archetypal example of a type II mutation where a nonfunctional protein is processed and secreted normally leading to a marked discrepancy between antigen and activity levels. A similar effect can be seen with mutations such as Arg43Gln and Arg46Ser in the propeptide (residues 29–46) which mediates the interaction with the vitamin K-dependent γ-carboxylase. Mutations in this region produce proteins with reduced function because of impaired phospholipid binding. The mechanism of the deficiency is the

same as that seen in vitamin K deficiency or warfarin therapy. Indeed, mutations in this region are also a well-known cause of increased sensitivity to coumarin anticoagulants. Many missense mutations are not quite so easily categorized because of both quantitative and qualitative effects. Nevertheless, this remains a useful classification, and a ratio of Ac:Ag of less than 0.7 is arbitrarily taken to indicate a predominantly type II defect. HB is unusual among coagulation factor deficiencies in that mutations are predominantly type II (approximately 60%). Most quantitative defects are caused by impairment of transcription or protein secretion. A reduction in circulating antigen may also be achieved by increased clearance of mutants with reduced stability. For many missense mutations the deficiency is caused by a combination of such mechanisms.

Nonsense mutations occur when a single nucleotide change results in the introduction of a premature stop codon. Generally, this results in a severe type I defect with no protein production. Thus, Gly357Arg and Gly357Glu are type II mutations with FIX:C <1 IU/dL and normal FIX:Ag but Gly357Ter is associated with undetectable antigen [19]. Occasionally, the mutation may be sufficiently near the C-terminus of the polypeptide to allow production of a truncated protein which may not have any function but contains an epitope recognized by an antigen assay. One possible mechanism by which normal protein can be detected despite a nonsense mutation is translational read-through. This is a poorly understood phenomenon that seems to be dependent on specific tRNA species that allow recognition of an incorrect stop codon and enable continued translation of the original open-reading frame.

Splicing of exons to form the correct RNA message relies on the recognition of splice junctions by RNA polymerases. Usually a pair of dinucleotides defines the splice donor and splice acceptor sites that instruct the polymerase to splice exonic sequences together. A single point mutation that destroys a splice donor site generally results in a nonsense mRNA because of destruction of the open-reading frame. Splicing defects (9% of all mutations) can also occur because of point mutations in intronic sequence that create an alternative splice acceptor site. The effects of this type of mutation are more difficult to predict because they depend on the probability of the polymerase choosing the alternative acceptor site over the correct one. Web-based software can be used to predict this probability using algorithms that take into account factors such as the similarity and proximity of the acceptor sites. Clear evidence of splicing mutations requires the detection of aberrant mRNA species but RT-PCR for HB presents technical difficulties, as indicated earlier.

Founder effect

The CpG effect does not explain all occurrences of mutation hotspots. Several groups have reported on the results of systematic mutation screening in a geographically restricted HB population. This has demonstrated a number of mutations that occur more frequently in the particular population under study but are not obviously explained by the previously discussed mechanisms. Further analysis of the surrounding region in the *F9* gene shows that polymorphic markers segregate with the mutation indicating the inheritance of a discrete rare haplotype. This is most likely to have arisen in a distant common ancestor or founder. If the gene pool from which the population arises remains relatively restricted, this allows the haplotype to become more prevalent. This effect is particularly seen in mild disorders where heterozygous individuals are largely unaffected.

Examples of mutations demonstrating the founder effect have been demonstrated in North America (Ile443Thr) [20], the UK (g.17781 A > G) [21], and Ireland (g.–35 G > A and Ala317Val) [22].

Hemophilia B Leyden

The abnormalities described above result from alterations of the coding sequence. Type I mutations can also arise from mutations affecting the gene's regulatory elements in the noncoding sequence (2% of all mutations). A well-characterized group of mutations affects the transcription factor binding sites in the *F9* promoter. This is in contrast to hemophilia A, where, thus far, only a single mutation in the *F8* promoter has been described. Figure 13.4 is a map of the relevant part of the *F9* promoter showing the known recognition sites of important transcription factors. Some mutations in this region

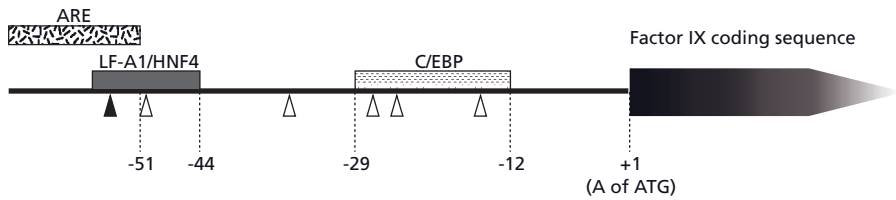

Figure 13.4 Map of the *F9* promoter showing the position of selected transcription factor-binding sites. ARE, androgen response element; LF-A1/HNF4, liver factor-A1/hepatocyte nuclear factor 4; C/EBP, CCAAT/enhancer binding protein. The dashed lines indicate the nucleotide number upstream of the transcription start site. White triangles indicate the position of the most common mutations associated with the Leyden phenotype. The black triangle indicates the position of a mutation that causes hemophilia B with stable levels throughout life.

can produce a standard type I phenotype with FIX levels at a stable low level throughout life. The HB Leyden phenotype is characterized by severe deficiency from birth with levels that start to increase during the second decade and achieve near-normality during the third decade [23]. Thereafter, normal or borderline-low levels are maintained throughout life. The cause of this phenomenon is apparent from the promoter map. This shows recognition sites for the transcription factors C/EBP and LF-A1/HNF that are active throughout life. One of these sites is partly overlapped by the androgen response element (ARE), which regulates transcription levels following the hormonal changes that occur during puberty. Mutations at g.–55 (such as the Brandenburg mutation) cause HB that persists throughout life because both the ARE and LF-A1/HNF sites are affected [24]. Mutations 3′ of g.–50 spare the ARE and cause HB Leyden because transcription is upregulated as testosterone levels increase.

Small insertions or deletions

Errors made by DNA polymerases generally result in point mutations. A rarer type of error is the insertion or deletion of a few nucleotides (18% of all mutations). Collectively, or where a single mutation results from a combination of such abnormalities, the term indels is used. These mutations often occur in sequences of dinucleotide repeats, particularly where one set of dinucleotide repeats is adjacent to a different set. Slippage of the DNA polymerase is one possible mechanism. As dinucleotide repeats are commonly intronic, such errors are frequently inconsequential. However, they can occasionally affect regulatory elements or lead to frame-shifts if coding sequence is involved. In the latter situation a severe deficiency is likely to result.

Gross genetic abnormalities

This type of abnormality includes gene rearrangements or deletions affecting the whole, or a large part, of the gene. These mutations are well represented in the literature because they are readily detected by methods based on DNA hybridization probes and invariably result in severe deficiency. However, they account for only 7% of HB cases in contrast with hemophilia A where gene rearrangements account for nearly half of severe cases (see Chapter 3). The inversion 22 rearrangement that is the commonest cause of severe hemophilia A occurs more frequently in the male gamete. This is thought to be because an additional X chromosome can protect against intrachromosomal rearrangement. On the other hand, deletions are more likely to be of female origin [25]. This may be a result of the chromosomal crossover that occurs during meiosis. As this process is dependent on recombination between homologous sequences, abnormal alleles are more likely to be of maternal origin because of the presence of two copies of the gene. Equivalent data in HB are lacking but as deletions are more common than inversions in *F9*, it may be that gross abnormalities are more likely to be of female origin.

From a scientific perspective a null allele gives us little information but the detection of these abnormalities often affects the clinical management of patients and relatives. Inhibitor formation is rare in HB (1–3% of all cases) compared with hemophilia A (about 30%) [26]. This is partly explained by the relatively low occurrence of gross gene deletions in HB. The probability of inhibitor formation is related to both severity of disease and type of genetic defect with the greatest risk associated with severe disease caused by gross genetic abnormalities. There are also differences in the clinical effects of inhibitors, with a number of reports of anaphylactic reactions. In relative terms these are less frequently seen in hemophilia A. Inhibitors in HB are discussed in greater length in the next chapter but it is clear that genetic factors play a role in both formation of inhibitors and the nature of the immune reaction.

Public databases

Haemophilia B Mutation Database: http://www.kcl.ac.uk/ip/petergreen/haemBdatabase.html

Coagulation Serine Protease Mutation Database: http://coagmdb.org/

The Human Gene Mutation Database: http://www.hgmd.cf.ac.uk/ac/index.php

Mutation nomenclature

Numbering in this chapter follows the guidelines of the Human Genome Variation (HGV) society. +1 refers to the "A" of the ATG translation start codon for nucleotide numbering and the methionine residue that this codes for in peptide numbering. Most earlier publications use a numbering based on the transcription start site or the mature protein.

References

1 Yoshitake S, Schach BG, Foster DC, Davie EW, Kurachi K. Nucleotide sequence of the gene for human factor IX (antihemophilic factor B). *Biochemistry* 1985; **24**: 3736–50.

2 Fay PJ. Activation of factor VIII and mechanisms of cofactor action. *Blood Rev* 2004; **18**: 1–15.

3 Brandstetter H, Bauer M, Huber R, Lollar P, Bode W. X-ray structure of clotting factor IXa: Active site and module structure related to Xase activity and hemophilia B. *Proc Natl Acad Sci USA* 1995; **92**: 9796–800.

4 Hopfner KP, Lang A, Karcher A, *et al*. Coagulation factor IXa: the relaxed conformation of Tyr99 blocks substrate binding. *Structure* 1999; **7**: 989–96.

5 Rao Z, Handford P, Mayhew M, Knott V, Brownlee GG, Stuart D. The structure of a Ca(2+)-binding epidermal growth factor-like domain: Its role in protein–protein interactions. *Cell* 1995; **82**: 131–41.

6 Huang M, Furie BC, Furie B. Crystal structure of the calcium-stabilized human factor IX Gla domain bound to a conformation-specific anti-factor IX antibody. *J Biol Chem* 2004; **279**: 14338–46.
7 Shen BW, Spiegel PC, Chang CH, *et al.* The tertiary structure and domain organization of coagulation factor VIII. *Blood* 2008; **111**: 1240–7.
8 Ngo JC, Huang M, Roth DA, Furie BC, Furie B. Crystal structure of human factor VIII: Implications for the formation of the factor IXa–factor VIIIa complex. *Structure* 2008; **16**: 597–606.
9 Autin L, Miteva MA, Lee WH, Mertens K, Radtke KP, Villoutreix BO. Molecular models of the procoagulant factor VIIIa–factor IXa complex. *J Thromb Haemost* 2005; **3**: 2044–56.
10 Bowen DJ. Haemophilia A and haemophilia B: Molecular insights. [erratum appears in *Mol Pathol* 2002; 55: 208.] [republished from *Mol Pathol* 2002; 55: 1–18.] *Mol Pathol* 2002; **55**: 127–44.
11 Green PM, Rowley G, Giannelli F. Unusual expression of the *F9* gene in peripheral lymphocytes hinders investigation of *F9* mRNA in hemophilia B patients. *J Thromb Haemost* 2003; **1**: 2675–6.
12 Sellner LN, Taylor GR. MLPA and MAPH: New techniques for detection of gene deletions. *Hum Mutat* 2004; **23**: 413–19.
13 Kwon MJ, Yoo KY, Kim HJ, Kim SH. Identification of mutations in the *F9* gene including exon deletion by multiplex ligation-dependent probe amplification in 33 unrelated Korean patients with haemophilia B. *Haemophilia* 2008; **14**: 1069–75.
14 Stenson PD, Ball E, Howells K, Phillips A, Mort M, Cooper DN. Human Gene Mutation Database: Towards a comprehensive central mutation database. *J Med Genet* 2008; **45**: 124–6.
15 Saunders RE, Perkins SJ. CoagMDB: A database analysis of missense mutations within four conserved domains in five vitamin K-dependent coagulation serine proteases using a text-mining tool. *Hum Mutat* 2008; **29**: 333–44.
16 Giannelli F, Green PM, Sommer SS, *et al.* Haemophilia B: Database of point mutations and short additions and deletions—eighth edition. *Nucleic Acids Res* 1998; **26**: 265–8.
17 Green PM, Saad S, Lewis CM, Giannelli F. Mutation rates in humans. I. Overall and sex-specific rates obtained from a population study of hemophilia B. *Am J Hum Genet* 1999; **65**: 1572–9.
18 Ljung RC, Sjorin E. Origin of mutation in sporadic cases of haemophilia A. *Br J Haematol* 1999; **106**: 870–4.
19 Wulff K, Bykowska K, Lopaciuk S, Herrmann FH. Molecular analysis of hemophilia B in Poland: 12 novel mutations of the factor IX gene. *Acta Biochim Pol* 1999; **46**: 721–6.
20 Thompson AR, Bajaj SP, Chen SH, MacGillivray RT. "Founder" effect in different families with haemophilia B mutation. *Lancet* 1990; **335**: 418.
21 Anagnostopoulos T, Morris AP, Ayres KL, Giannelli F, Green PM. DNA variation in a 13-Mb region including the *F9* gene: Inferring the genealogical history and causal role of a hemophilia B mutation (IVS 5 + 13 A → G). *J Thromb Haemost* 2003; **1**: 2609–14.
22 Jenkins PV, Egan H, Keenan C, *et al.* Mutation analysis of haemophilia B in the Irish population: Increased prevalence caused by founder effect. *Haemophilia* 2008; **14**: 717–22.
23 Ljung R, Nilsson IM, Hemophilia B Leyden and a similar variant of hemophilia A. *N Engl J Med* 1982; **307**: 897–8.
24 Crossley M, Ludwig M, Stowell KM, De VP, Olek K, Brownlee GG. Recovery from hemophilia B Leyden: An androgen-responsive element in the factor IX promoter. *Science* 1992; **257**: 377–9.
25 Becker J, Schwaab R, Moller-Taube A, *et al.* Characterization of the factor VIII defect in 147 patients with sporadic hemophilia A: Family studies indicate a mutation type-dependent sex ratio of mutation frequencies. *Am J Hum Genet* 1996; **58**: 657–70.
26 DiMichele D. Inhibitor development in haemophilia B: An orphan disease in need of attention. *Br J Haematol* 2007; **138**: 305–15.

14 Factor IX inhibitors in hemophilia B

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Frequency of inhibitors in hemophilia B

Factor (F) IX inhibitors are relatively uncommon, being seen in 1–3% of persons with hemophilia B. In fact, the long-term directors of some large hemophilia centers note that they have seen no FIX inhibitors in their centers (Table 14.1). This is in striking contrast to the situation in hemophilia A, where approximately 30% of individuals with severe and moderately severe hemophilia develop inhibitors to FVIII [1,2]. Among persons with hemophilia, approximately 80% have hemophilia A, while only 20% have hemophilia B. Hemophilia B, an X-linked recessive disorder, occurs in approximately one of 30 000 male births, in all populations. Mutations causing this bleeding disorder have been found all over the FIX gene, which is located at Xq27.1; these mutations are reported and updated in the *F9* Mutation Database (http://www.kcl.ac.uk/ip/petergreen/haemBdatabase.html) [3]. Based on the measurable activity of the patient's FIX, hemophilia B is classified as severe (<1%), moderate (1–5%), or mild (>5%); in most reports, approximately 30–45% of affected individuals have severe hemophilia B [4]. The majority of persons with hemophilia B who develop inhibitors have severe hemophilia B.

Risk factors for development of factor IX inhibitors

Genetic factors play a major role. Certain mutations in the FIX gene are associated with an increased incidence of inhibitor development. Large deletions and frame-shift mutations leading to the loss of coding information are much more likely to be associated with inhibitor development. High has reported that large deletions account for only 1–3% of all hemophilia B patients, but account for 50% of inhibitor patients [5]. In an analysis of hemophilia B patients who had anaphylactic reactions to FIX-containing products, as well as an inhibitor, Thorland and coworkers genotyped eight unrelated patients and compared their gene mutations with those found in 550 hemophilia B patients in the hemophilia B database at the time. Individuals with complete gene deletions were found to be at greatest risk for anaphylaxis. Anaphylaxis occurred more frequently in families with null mutations (large deletions, frame-shift, or nonsense mutations) than in those with missense mutations [6].

In addition to the particular FIX defect causing a patient's hemophilia B, Astermark has recently reviewed the potential role of immune response genes (noting a microsatellite polymorphism in the promoter region of the IL-10 gene, which was highly associated with inhibitor formation in hemophilia A patients), environmental factors, and other concurrent immune system challenges, among others [7,8].

While race has been shown to play a definite role in the development of FVIII inhibitors, with approximately 40–50% of black individuals with hemophilia A developing a FVIII inhibitor, no such association has been found in hemophilia B.

Age and number of exposure days to fix at detection of factor IX inhibitors

As is the case in hemophilia A, most (but not all) individuals who develop an inhibitor to FIX do so relatively early in life (within the first 4–5 years), after a median of 9–11 exposure days (EDs) to any FIX-containing product (Table 14.2).

Anaphylaxis and other allergic reactions developing in close association with factor IX inhibitor development

With the development of plasma-derived FIX products of higher purity (e.g., monoclonal antibody purified FIX concentrates) in the 1990s, prelicensure clinical trials revealed an occasional study subject who developed anaphylaxis while being infused with the higher purity product [2]. This raised concern that such high-purity products, which perhaps had been altered in their production, were resulting in both anaphylaxis and FIX inhibitors. As a result, an international registry of such complications was organized by Warrier *et al.*, on behalf of the FVIII/FIX Subcommittee of the International Society on Thrombosis and Hemostasis (ISTH)'s

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Table 14.1 Reasons for lower numbers of factor IX (FIX) inhibitors than factor VIII (FVIII) inhibitors.

Fewer numbers of individuals with hemophilia B as compared with hemophilia A
Lower percentage of persons with severe hemophilia B (most inhibitors in hemophilia B occur in severely affected individuals, i.e., those with <1% FIX)
FIX is one-fifth the size of FVIII, and distributes extravascularly
Higher FIX (than FVIII) protein (5 μg/mL vs. 100 ng/mL)
Structural analogy to other vitamin K-dependent factors may confer some tolerance to FIX

Table 14.2 Demographic data for individuals with factor IX (FIX) inhibitors.

Median age at detection of FIX inhibitors: 19.5 months
Median exposure days (EDs) at detection of FIX inhibitors: 9–11 (range 2–180 EDs)
Median peak inhibitor titers: 30 BU (1–1156 BU)
Occur in all racial and ethnic groups, with no predilection for a certain race
Most occur in persons with null mutations in the FIX gene (large gene deletions, frame-shift mutations)
Roughly one-half of patients with hemophilia B and a FIX inhibitor have anaphylactic (or severe allergic) reactions to any FIX-containing product

Scientific and Standardization Committee (SSC) [4,9,10]. Additionally, all subsequent prelicensure clinical trials with new products included FIX inhibitors assays done at more frequent (specified) time intervals, and over a longer period of time.

Interestingly, the ISTH registry data submitted from 1997 to 2006 provided data concerning 94 individuals with inhibitors and anaphylaxis or severe reactions to FIX-containing products of various types. Some were receiving intermediate-purity plasma-derived products, while others were receiving high-purity FIX products (either recombinant or plasma-derived) when the reaction occurred. Thus, there was no evidence to implicate a particular type of product [4].

However, in view of the severity of such complications occurring early in life, after very few exposures to FIX, Warrier *et al.* recommended that all infants and small children with severe hemophilia B be closely followed over their first 20 (or more) infusions (with any FIX-containing product) in a facility equipped to treat anaphylactic shock [9–11]. It was also recommended that genotyping be done on infants (or an affected sibling) with severe hemophilia B before such complications occurred, to know if they had a defect in the FIX gene (e.g., a large gene deletion) putting them at particular risk for anaphylaxis and inhibitor development [10].

In general, patients with an inhibitor to FIX and a history of anaphylaxis to FIX should be treated with rFVIIa—a safe, readily available recombinant product which does not contain FIX.

Management of patients with hemophilia B complicated by a factor IX inhibitor

As mentioned above, infants and young children with severe hemophilia B are at particular risk for the sudden development of anaphylactic shock (or other severe allergic reaction) and inhibitor development. While these two events are often closely related temporally, one may precede the other. Thus, a child who has developed a FIX inhibitor after relatively few EDs to FIX should be regarded as being at greater risk for anaphylactic shock with one of his next several doses of FIX. While such children are often being treated at a hemophilia treatment center (i.e., not yet on home infusion), the center should be equipped with a readily accessible "crash cart" for treating patients in shock.

Once stabilized, and with the situation being discussed with the parents, parents and members of the hemophilia center team can *consider* attempting desensitization with gradually increased doses of FIX. If this is successful, one can then *consider* an immune tolerance induction (ITI) regimen, using daily (or every other day) larger doses of FIX. However, while ITI regimens are often (approximately 85% of the time) successful in persons with hemophilia A complicated by a FVIII inhibitor, the success rate is much lower in hemophilia B. An average success rate here would be 40%, and ITI is fraught with complications. If success is achieved, it takes longer. More importantly, many hemophilia B patients undergoing ITI develop nephrotic syndrome approximately 7–8 months into ITI [9,11–13]. Should this occur, ITI should be stopped; stopping FIX has resulted in improvement or cessation of edema and proteinuria in some reported patients [4]. In two patients who had developed nephrotic syndrome while on ITI, renal biopsy demonstrated membranous glomerulonephritis [12–14].

Management of bleeding episodes in patients with factor IX inhibitors

The mainstays of treatment (or prevention) of bleeding episodes in individuals with hemophilia B complicated by an inhibitor to FIX are rFVIIa (Novo Nordisk's NovoSeven®), and Baxter's FEIBA-VH. Each has advantages and disadvantages [15–18]. rFVIIa is a recombinant product, and thus safe from transmission of blood-borne infections. It has been licensed for use in European countries and in North American for approximately a decade, and has generally proven to be safe and effective [16–17]. It can be safely used at home. However, there is no simple, readily available laboratory test for evaluating or predicting its efficacy, and the product is quite expensive [15,16,19]. While the package insert in the USA indicates a dosage range of 90–120 μg/kg per dose, to be

given every 3 h if repeat doses are necessary, many hemophilia treaters are now using higher doses (e.g., 270 μg/kg/dose), especially in children and young adults. Anecdotal reports indicate success with a single dose in certain situations, with no adverse effects [20,21].

FEIBA-VH has also proven effective in many (but not all) bleeding situations. It is generally less expensive than rFVIIa, and if additional doses are needed, they are generally given at 12-h intervals. However, it is a plasma-derived product, and thus some worry about its safety, despite its track record of safety over many years of use. Some patients have hives and pruritis with its use, and thus have been switched to rFVIIa.

Several studies comparing the above two products (FENOC study), or comparing different doses of rFVIIa, are ongoing or near completion, and anecdotal reports describe the efficacy of rituximab in small numbers of difficult-to-manage FIX inhibitor patients [21–23].

References

1 Lusher J. Natural history of inhibitors in severe hemophilia A and B: incidence and prevalence. In: Rodriguez-Merchan EC, Lee CA (eds.) *Inhibitors in Patients with Haemophilia*. Oxford: Blackwell Science Ltd., 2002: 3–8.

2 Lusher JM. Inhibitors in young boys with haemophilia. *Baillieres' Clin Haematol* 2000; **3**: 457–68.

3 Bicococchi M, Pasino M, Rosano C, *et al.* Insight into molecular changes of the FIX protein in a series of Italian patients with haemophilia B. *Haemophilia* 2006; **12**: 263–270.

4 Chitlur M, Warrier I, Rajpurkar M, Lusher JM. Inhibitors in factor IX deficiency a report of the ISTC-SSC international FIX inhibitor registry (1997–2006). *Haemophilia* 2009; **15**: 1027–31.

5 High HA. Factor IX molecular structure, epitopes and mutations associated with inhibitor formation. In: Aledort LM, Hoyer LW, Lusher JM, *et al.* (eds.) New York: Plenum Press, 1995: 79–86.

6 Thorland ED, Drost JB, Lusher JM, *et al.* Anaphylactic response to factor IX replacement therapy in haemophilia B patients: Complete gene deletions confer the highest risk. *Haemophilia* 1999; **5**: 101–5.

7 Astermark J. Basic aspects of inhibitors to factors VIII and IX and the influence of non-genetic risk factors. *Haemophilia* 2006; **12**(Suppl. 6): 8–14.

8 Astermark J. Why do inhibitors develop? Principles of and factors influencing the risk for inhibitor development in haemophilia. *Haemophilia* 2006; **12**(Suppl. 3): 52–60.

9 Warrier I. ITI in hemophilia B: Possibilities and problems. The International Monitor on Hemophilia. 10 Year Anniversary Issue, 2003: 20–23.

10 Warrier I, Ewenstein B, Koerper M, *et al.* FIX inhibitors and anaphylaxis in hemophilia B. *J Pediatr Hematol Oncol* 1997; **19**: 23–7.

11 Warrier I. Management of haemophilia B patients with inhibitors and anaphylaxis. In: Varon D, Martinowitz U, Heim M (eds.) *Haemophilia and Related Disorders. Haemophilia, volume 4*. Oxford: Blackwell Science Ltd., 1998: 574–6.

12 Ewenstein BM, Takemoto C, Warrier I, *et al.* Nephrotic syndrome as a complication of immune tolerance in hemophilia B. *Blood* 1997; **89**: 1115–16.

13 Dharnidharka VR, Takemoto C, Ewenstein BM, *et al.* Membranous glomerulonephritis and nephrosis post factor IX infusions in hemophilia B. *Pediatr Nephrol* 1998; **12**: 654–7.

14 Warrier I. Factor IX inhibitors and anaphylaxis. In: Rodriguez-Merchan ED, Lee CA (eds.) *Inhibitors in Patients with Haemophilia*. Oxford: Blackwell Science Ltd., 2002: 87–91.

15 Astermark J, Rocino A, von Depka A M, *et al.* Current use of bypassing agents in Europe in the management of acute bleeds in patients with haemophilia and inhibitors. *Haemophilia* 2007; **13**: 38–45.

16 Sumner MJ, Geldziler G, Pedersen M, *et al.* Treatment of acquired haemophilia with recombinant activated FVIIa: A critical appraisal. *Haemophilia* 2007; **13**: 451–61.

17 Hedner U. Products used to treat hemophilia: Recombinant factor VIIa. In: Lee CA, Berntorp EE, Hoots WK (eds.) *Textbook of Hemophilia*, Vol. **2**. Oxford: Blackwell Publishing, 2007: 147–152.

18 Brown SA. Treatment of inhibitors in hemophilia B. In: Lee CA, Berntorp EE, Hoots WK (eds.) *Textbook of Hemophilia*, Vol. **1**. Oxford: Blackwell Publishing, 2007: 101–5.

19 Lacy L. Economic impact of treating inhibitor patients. *Pathophysiol Haemost Thromb* 2002; **32**(Suppl. 1): 29–32.

20 Kenet G, Lubetsky A, Luboshitz J, *et al.* A new approach to treatment of bleeding episodes in young hemophilia patients: A single bolus megadose of recombinant activated factor VII (NovoSeven). *J Thromb Hemost* 2003; **1**: 450–5.

21 Parameswaran R, Shapiro AD, Gill JC, *et al.* Dose effect and efficacy of rFVIIa in the treatment of haemophilia patients with inhibitors: Analysis from the Hemophilia and Thrombosis Research Society Registry. *Haemophilia* 2005; **11**: 100–6.

22 Fox RA, Neufeld EJ, Bennett CM. Rituximab for adolescents with haemophilia and high titre inhibitors. *Haemophilia* 2006; **12**: 218–22.

23 Lusher JM. Rituximab and desensitization for a patient with factor IX deficiency (review). *The International Monitor on Hemophilia* 2008; **2**: 38–40.

15 Treatment of inhibitors in hemophilia B

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Introduction

Inhibitor development continues to be one of the most significant complications of hemophilia B [factor IX (FIX) deficiency], resulting in an increased risk of morbidity and mortality. Owing to the relative rarity of both FIX deficiency and inhibitor development, the ability to establish evidence-based treatment approaches has been limited. Treatment of individuals with FIX deficiency complicated by inhibitors may be divided into two categories: Those strategies aimed at treatment and/or prevention of acute bleeding, and those directed toward inhibitor eradication. Therapeutic interventions to accomplish these goals require in-depth knowledge of the use of bypassing agents, and the potential risks and overall success rate associated with presently available methods of immune tolerance therapy. Inhibitor development in hemophilia B is associated with specific risks well documented in the literature, including a wide spectrum of infusion reactions ranging from mild to life-threatening. In addition, immune tolerance has a decreased overall success rate compared with this therapy in factor VIII (FVIII) deficiency and may be complicated by unique sequelae including recrudescence of infusion reactions and nephrotic syndrome. Recombinant activated factor VII (rFVIIa) has emerged as an important therapy in patients with infusion reactions for the treatment or prevention of hemorrhage while new innovative strategies aimed at inducing immune tolerance require further research.

Epidemiology

Approximately 3–4% of patients with severe FIX deficiency develop inhibitors [1,2]. Inhibitor development most often occurs early during life and commonly within the first 30 exposure days to exogenous FIX, with a reported median of 11 exposure days [3]. These data are consistent with inhibitor development in FVIII deficiency. However, in contrast to FVIII deficiency, the risk of inhibitor development in hemophilia B appears to be spread equally across all races; in addition, inhibitors in hemophilia B do not appear to be influenced by specific FIX replacement products including plasma derived or recombinant [4]. Inhibitors in FIX deficiency are generally limited to patients with severe disease and are extremely rare in mild and moderate variants [1] (Table 15.1). Development of neutralizing antibodies to FIX is frequently heralded by the appearance of infusion-associated reactions [4]. Approximately 80% of FIX inhibitors are high responding, characterized by an antibody titer ≥5 Bethesda units (BU) at any historical time point, and a strong anamnestic response [2].

Potential explanations for the lower incidence of inhibitors in hemophilia B compared with hemophilia A, where the incidence approaches 30%, include a lower proportion of severe phenotype among hemophilia B patients [5], higher proportion of the presence of antigenic FIX termed cross-reactive material positive (CRM+) [6], decreased immunogenicity because of homology of FIX to the other vitamin K-dependent clotting factors [4], and increased presence of missense mutations causative for severe disease; approximately 60% of severe FIX deficiency results from missense mutations [7] providing an explanation for the high rate of CRM positivity and thus the lower incidence of inhibitors in hemophilia B.

Genetic and other risk factors of inhibitor development

The importance of genetic risk factors in inhibitor development has been implicated in several reports. The Malmö International Brothers Study (MIBS) reported a high concordance rate among siblings with inhibitors. However, this study included only four families with hemophilia B with inhibitors of which two families had more than two siblings with inhibitors [8]. The relative infrequency of FIX inhibitors makes it difficult to draw significant conclusions about the contribution of risk factors such as race or environmental influences. The genetic defects associated with highest risk of inhibitor formation in FIX deficiency most commonly include large gene abnormalities such as major deletions and nonsense mutations resulting in absence of FIX protein [9]. Thus, genetic analysis at birth in individuals without a family history is important to identify those at risk for this serious sequela. Environmental

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Table 15.1 Comparison of inhibitors in hemophilia A and B.

Area	Inhibitors in factor VIII deficiency	Inhibitors in factor IX deficiency
Inhibitor development in severe disease	High incidence—approximately 20–30% of patients develop inhibitors	Low incidence—approximately 2–4% of patients develop inhibitors
Inhibitor development in moderate to mild disease	Between 3% and 13% of patients develop inhibitors	Extremely rare in mild to moderate hemophilia B
Impact of race	Increased incidence in African-Americans	No conclusive evidence of racial difference in incidence
Presence of infusion-associated reactions	Infusion reactions rare	Approximately 60% of patients with inhibitors have associated infusion reactions when exposed to exogenous factor IX
Success of immune tolerance	Successful in 60–70% of patients	Difficult to achieve with only 15–30% reported success rate
Complications of immune tolerance	Well tolerated	Nephrotic syndrome well-documented complication, especially in patients with history of infusion reactions

risk factors reported to contribute to inhibitor development in FVIII deficiency, including age at first exposure, concurrent inflammatory states including infection, type of replacement therapy utilized, and mode of administration (bolus versus continuous infusion) have been debated but not definitely demonstrated to play a significant role in inhibitors in FIX deficiency.

Immunology

Inhibitors to FIX are neutralizing antibodies that rapidly bind and inhibit FIX activity, making the achievement of a hemostatic level through exogenously administered FIX concentrate difficult if not impossible. The most common epitopes to which inhibitory antibodies bind reside within the γ-carboxyglutamic acid region and the serine protease domain of the FIX molecule [10] (Plate 15.1).

Inhibitory antibodies are most often polyclonal and predominantly IgG4, although complement binding IgG1 subclass appearing transiently during anaphylactoid episodes has been reported. Rarely, IgA antibodies have been demonstrated [11]. Although IgE antibodies have been hypothesized [12] to occur in patients with infusion reactions to FIX as a result of the presence of symptoms of bronchospasm and hypotension, these have not been convincingly demonstrated [13]. Therefore, these reactions are most appropriately termed "anaphylactoid" rather than anaphylactic because of the absence of a well-defined IgE-mediated mechanism.

Approximately 60% of factor IX inhibitors are associated with reactions to exposure to exogenous FIX, ranging from mild symptoms of watery suffused eyes to severe hypotension and bronchospasm [4]. Despite the lack of an associated IgE antibody, desensitization protocols have been undertaken and proven to be of benefit to ameliorate or abate these reactions [12].

Inhibitor presentation

The development of an inhibitor in individuals with hemophilia is often suspected because of lack of, or poor response to, infusion therapy for treatment of a bleeding episode. The development of unusual symptoms related to infusions, including watery suffused eyes, cough, bronchospasm, and hypotension, should raise suspicion and is a reason to intiate testing. In addition, routine inhibitor surveillance testing may be positive in the absence of the above symptoms. In patients with a documented family history of FIX deficiency complicated by inhibitors, routine surveillance and close clinical monitoring should be instituted in all subsequently affected individuals because of the increased risk.

Treatment strategies

As FIX deficiency is a rare disorder and the frequency of inhibitor development is <5% in severe disease, the number of individuals with this complication predicted in the USA is quite small (approximately 100). The paucity of patients has resulted in lack of evidence-based standardized strategies for both treatment or prevention of bleeding episodes and inhibitor eradication.

Control of acute bleeding

Treatment of hemorrhagic episodes in patients with FIX deficiency complicated by inhibitors is dependent upon the type of bleeding episode experienced, the inhibitor classification (high- versus low-responding), the history and severity of infusion reactions, and in some circumstances the inhibitor titer. Inhibitors that are classified as low-responding are those with a persistent level of <5 BU despite repeated exposure. Patients with low-responding inhibitors in the absence of infusion reactions may be treated with doses of FIX concentrate calculated to overcome the inhibitor titer and achieve a hemostatic level. Monitoring of FIX activity levels may be required to assure achievement of a hemostatic level for a sufficient period of time to allow cessation and/or prevention of bleeding, and

healing to occur. Unfortunately, the number of low-responding inhibitor patients in hemophilia B constitutes a minority of the affected population.

In patients with high-responding inhibitors, either the inhibitor titer or anamnestic response make the use of FIX concentrates for treatment impractical. As this is the population of patients who most commonly experience infusion-associated reactions, exposure to FIX-containing concentrates should only be considered by care-givers who have a detailed knowledge of the patient's medical history and experience in treatment of these most difficult patients. In times of life- or limb-threatening bleeding, use of FIX concentrate may be considered based upon the patient's response to bypassing therapy, their history of infusion-associated reactions, and their present inhibitor titer. A history of infusion-associated reactions makes pretreatment and careful observation in a highly controlled environment necessary if this approach is utilized.

The most commonly utilized therapy for hemostatic control in patients with high responding inhibitors with FIX deficiency and a history of infusion reactions is activated recombinant factor VII concentrate (rFVIIa; NovoSeven®, Novo Nordisk, Bagsvaerd, Denmark). Standard dosing regimens of rFVIIa include doses of 90–120 μg/kg/dose administered every 2–3 h until control of bleeding has been achieved. Use of plasma-derived activated prothrombin complex concentrates (aPCCs; FEIBA® VH, Baxter Healthcare Corporation) may be utilized in these patients if they do not experience infusion reactions; aPCCs contain FIX and therefore caution should be utilized in this patient population. Repeated exposure to products containing FIX even in the absence of infusion-associated reactions will serve to stimulate the inhibitor titer and prevent its natural decline over time. Therefore, patients who utilize aPCCs with FIX deficiency and inhibitors are likely not to achieve a low-titer inhibitor, which in times of life- or limb-threatening bleeding unresponsive to rFVIIa, or in which rFVIIa is felt to pose a risk, will not have FIX concentrate as a potential available therapy. Common dosing strategies for FEIBA® range from 50 to 100 units/kg/dose at intervals of approximately 12 h, with a daily dose not to exceed 200 units/kg/day. FEIBA® VH is a plasma-derived product and carries a small risk of blood-borne viral infection, although none have been reported in over 30 years [14]. For high-responding inhibitor patients with allergic manifestations to factor IX infusions, rFVIIa is clearly the treatment of choice [15]. The majority of patients with FIX deficiency complicated by inhibitors continue to be treated at home.

The safety and efficacy of both aPCCs and rFVIIa for home treatment are well documented in the literature [16,17]. The response rate of bleeding episodes to these agents varies by report, by type of bleeding event treated, and by dosing regimens utilized [18,19]. In addition, response rates may also vary based upon the definition utilized for a successful treatment outcome. The more rigorous clinical trials have utilized more rigid definitions of success and tend to report overall lower success rates [20]. The overall response rates for musculoskeletal hemorrhage have been reported in the range of 80–90%. In a minority of patients, response to one particular agent or in a particular bleeding event may be poor, necessitating alteration of the administration regimen or product utilized. The use of aPCC in these patients must be based upon a individual patient's history of infusion-associated reactions and their response to therapy for a bleeding event [21].

Adverse events have been reported to occur with both aPCC and rFVIIa. With aPCC, thrombotic events, including unusual episodes of transmural cardiac ischemia, have been documented [22]. Adverse events have also been reported to occur with rFVIIa, although the majority has occurred in its off-label use outside of treatment of hemophilic patients with inhibitors [23,24]. rFVIIa has a wide safety margin and successful use of doses up to 240–320 μg/kg without apparent change in the adverse event profile has been increasingly reported [25]. High-dose therapy allows for an increased dosing interval, and a theoretically improved thrombin burst and subsequent clot stability [26]. Higher doses may also be required in subsets of patients with increased clearance, as in the pediatric population [27,28].

Dosing strategies should include early institution of therapy as it has been documented that therapy administered earlier in the course of a bleeding event is associated with an increased rate of clinical response and decreased use of replacement therapy [17,29].

Sequential dosing of aPCC and rFVIIa has been rarely utilized in unresponsive bleeding episodes in a controlled setting such as hospitalized patients. The risk of adverse events including thrombosis may be increased with this approach necessitating close clinical and laboratory monitoring; this approach should be undertaken only by healthcare providers with specific expertise in this area and for patients who have failed all other interventions [30]. In patients with inhibitor titers greater than 10 BU who experience life-threatening bleeding episodes unresponsive to bypassing therapy where use of FIX concentrate would be considered life- or limb-saving, immunoadsorption with staphylococcal protein A or plasmapheresis to acutely lower the antibody titer and allow use of FIX concentrate may be considered.

Prophylaxis

Hemophilia patients with inhibitors have traditionally been treated with on-demand therapy for bleeding episodes resulting in an increased risk of musculoskeletal complications and decline in health-related quality of life (HRQoL) compared with patients without inhibitors, especially those treated with a prophylactic infusion regimen [31]. Within the last decade, case reports documented that bypassing agents may be utilized in patients with inhibitors on a prophylactic basis to suppress the number of bleeding events [32]. Subsequently, a prospectively conducted controlled trial was performed utilizing rFVIIa for secondary prophylaxis in hemophilic patients with

inhibitors that documented a decreased rate of bleeding with daily administration. Two dose levels were utilized, 90 μg/kg/dose compared with 270 μg/kg/dose, with a decrease in bleeding events in the higher-dose group that was not statistically significant, most likely related to the small number of patients included in the trial (22 patients with inhibitors; 21 with hemophilia A and one with hemophilia B) [33]. A trial is presently under way evaluating the prophylactic use of aPCC in inhibitor patients [34]. As musculoskeletal disease has been documented to be inversely associated with HRQoL [35], efforts to provide improved outcome and decrease bleeding events in inhibitor patients is rationale and warranted.

Overview of immune tolerance

As patients with inhibitors suffer increased risk of poorly controlled hemorrhagic episodes that impact an individual's musculoskeletal outcomes and HRQoL, inhibitor eradication through immune tolerance is logical to simplify treatment, potentially allow for prophylactic therapy, and improve patient outcome. Immune tolerance was first described by Brackmann in 1984 in a patient with FVIII deficiency and an inhibitor [36]. Since this first report, subsequent case reports and series appeared using similar or modified protocols demonstrating the effectiveness to varying degrees of immune tolerance for inhibitor eradication [37,38]

In 1981, Dr. Nilsson *et al.* reported the use of immunomodulation (cyclophosphamide and intravenous immunoglobulin) and immunoadsorption when inhibitor titers were greater than 10 BU, in addition to use of intensive replacement therapy for immune tolerance induction in a patient with FVIII inhibitor; this regimen was subsequently termed the Malmö protocol [39]. Between 1980 and 1995, approximately five patients in Sweden with hemophilia B and high-responding inhibitors were treated successfully using this regimen [40]. Despite initial enthusiasm [41], this strategy has not been shown to provide an increased rate of efficacy or longevity of overall tolerance; because of the use of immunomodulatory agents and lack of data supporting increased success, this regimen has been largely abandoned [4,42].

Unfortunately, the majority of work in the area of immune tolerance relates to FVIII deficiency. The overall reported success of immune tolerance regimens varies within the literature. Despite an estimated overall success rate in FVIII deficiency of approximately 75%, the rate of reported success of these protocols in FIX deficiency with inhibitors is far less (approximately 25–35%) [4,42].

Immune tolerance induction in hemophilia B

Early experience utilizing the Malmö protocol in FIX deficiency with inhibitors was encouraging, with reported success rates approaching 85%; however, these data represented only seven patients, of which six achieved sustained tolerance. [41]. Further data did not support this success rate and in fact revealed a far poorer outcome (56%) of immune tolerance protocols in FIX deficiency compared with FVIII [43].

Overall success rates of immune tolerance in FIX deficiency have varied. The North American Immune Tolerance Registry reported 31% [42] whereas the experience with the International Immune Tolerance Registry was even lower at 15% [4]. There are unique challenges in immune tolerance in patients with FIX deficiency and inhibitors even beyond the poor outcome. Patients with the allergic phenotype require desensitization so as to be able to utilize FIX therapy required in immune tolerance regimens. A successful desensitization protocol involving skin testing to FIX-containing products followed by infusion of the same with gradual dose escalation and continuous infusion over several hours has been described by Dioun *et al.* [12]. A similar protocol with the addition of rituximab has been recently described in a case report where immune tolerance was achieved [44].

The development of nephrotic syndrome in patients with hemophilia B on immune tolerance was first reported by Ewenstein *et al.* [45]. Although the pathophysiology of this sequela has not been definitively elucidated, one case report has demonstrated immune complex deposits within the basement membrane consistent with membranous glomerulonephritis [46], yet this finding has not been consistently substantiated in further reports. Thirteen patients in the international immune tolerance registry (ITI) have developed nephrosis, of which 11 patients (85%) had infusion reactions to FIX. Renal biopsy, performed in two patients, showed membranous glomerulonephritis. However, immunohistochemical staining undertaken in one patient failed to show FIX-containing immune complexes [4]. Nephrosis typically occurs 8–9 months into therapy and is often associated with the allergic phenotype. Nephrosis in these patients is dissimilar to childhood nephrosis as it is steroid nonresponsive and requires withdrawal of the antigenic stimulus, FIX, for resolution [47].

Because of the poor success rate with standard immune tolerance regimens in hemophilia B, new strategies were employed in an effort to achieve improved outcome. Anecdotal success has been reported with cyclosporine A [48], mycophenolate mofetil [49], and more recently with rituxan [50]. The experience with any one of these agents is quite limited and generalized recommendations are not possible. Clearly, this subset of patients is in need of novel treatment regimens to achieve an improved outcome.

Acquired inhibitors in nonhemophilic patients

Acquired inhibitors to FIX are rare, anecdotally reported, and often associated with an underlying immunologic disorder

such as systemic lupus erythematosus, hepatitis, multiple sclerosis, rheumatic fever, and collagen vascular disorders [51]. Treatment of hemorrhagic episodes is dependent on bypassing agents. A variety of immunosuppressive agents including steroids have been utilized for inhibitor eradication with variable response. Mazzucconi *et al.*, from Italy, reported a case of a 2-year-old child who developed an acquired FIX inhibitor successfully treated with intravenous immunoglobulin and high-dose steroids. [52]. A similar case was reported by Miller, successfully treated with plasma exchange, cyclophosphamide, and steroids [53]. Ozsoylu *et al.*, from Turkey, reported a 14-year-old child and a 31-year-old adult with acquired inhibitors to FIX, both responsive to high-dose steroids [54]. FIX inhibitors in association with colon carcinoma have been reported and were steroid responsive [55]. Lecumberri *et al.* reported a case of postpartum FIX acquired inhibitor where hemostasis was achieved with rFVIIa; the inhibitor was transient and steroid responsive [56]. Acquired FIX inhibitor kinetics have not been well delineated. Acquired inhibitors may be transient and/or respond to immunosuppression. Hence, it appears from the literature that a conservative approach to treating these patients may be utilized and that rFVIIa has been useful in achieving hemostasis.

Conclusion

Treatment of inhibitors in hemophilia B may be divided into two approaches: The first aimed at securing hemostasis during an acute bleeding episode and/or surgical procedure and the second aimed at inhibitor eradication through immune tolerance. Treatment of bleeding episodes is dependent upon knowledge of a variety of factors including the type of bleeding episode, type of inhibitor (low- vs. high-responding), and the presence of an allergic phenotype (Figure 15.1). Genetic analysis in sporadic cases is important to evaluate risk of inhibitor development. Exposure to exogenous FIX concentrate should be performed in a controlled setting in patients whose individual or family mutation is unknown until the time of highest risk has passed, minimally considered being the first 20 exposure days. The family and care-giver should be aware of the signs and symptoms of inhibitor development and serial monitoring should be performed during the first 20 exposure days or when an inhibitor is suspected because of symptoms. Individuals with FIX deficiency and inhibitors are an unusual subset of patients who require a knowledgeable care-giver and staff to achieve optimal outcome; these patients are best managed at hemophilia treatment centers with expertise in this area.

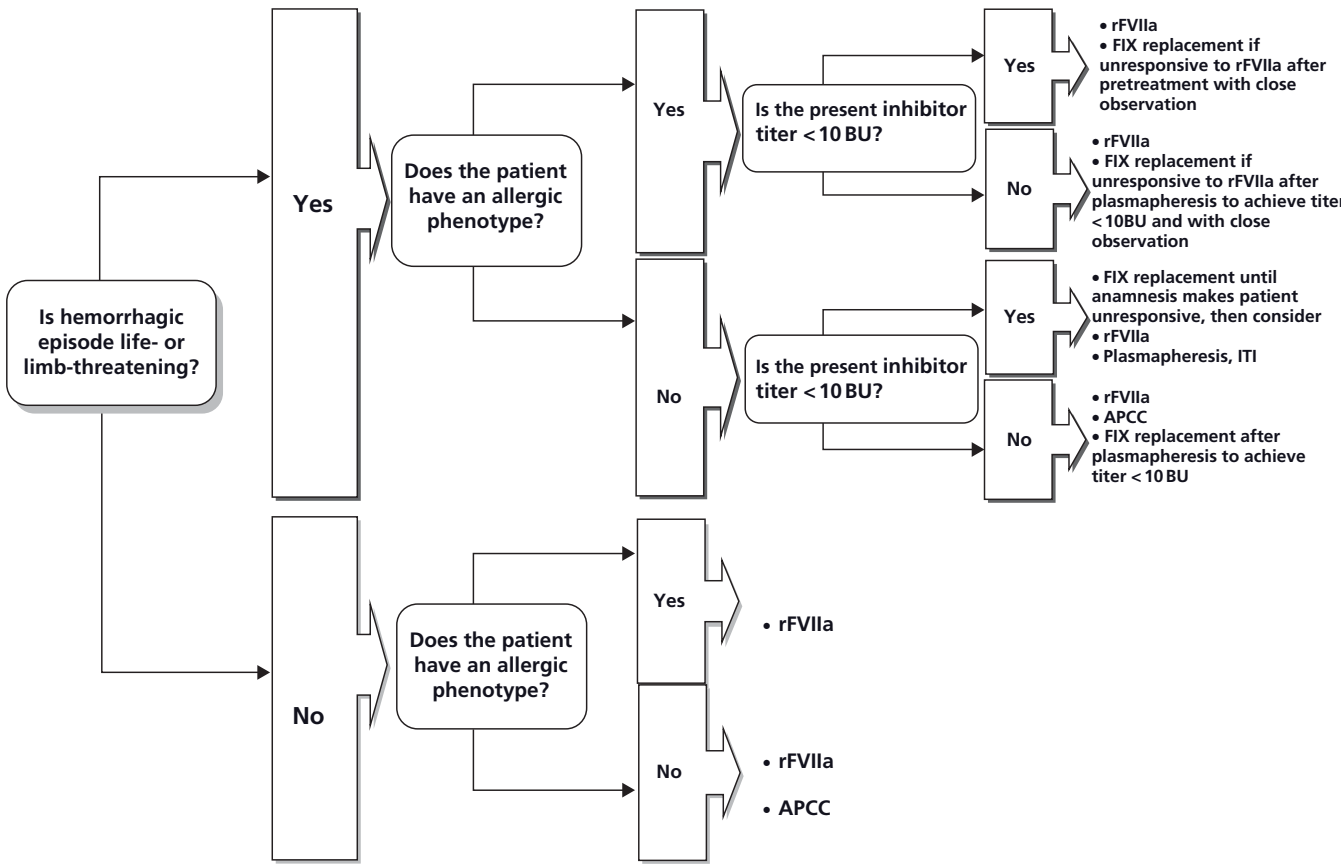

Figure 15.1 Algorithm for treatment of patients with high-responding factor IX deficiency and inhibitors for acute bleeding episodes.

References

1 Sultan Y. Prevalence of inhibitors in a population of 3435 hemophilia patients in France. French Hemophilia Study Group. *Thromb Haemost* 1992; **67**: 600–2.

2 Key NS. Inhibitors in congenital coagulation disorders. *Br J Haematol* 2004; **127**: 379–91.

3 Warrier I, Ewenstein BM, Koerper HA, *et al*. Factor IX inhibitors and anaphylaxis in hemophilia B. *J Pediatr Hematol Oncol* 1997; **19**: 23–7.

4 Chitlur M, Warrier I, Rajpurkar M, et al. Inhibitors in factor IX deficiency a report of the ISTH-SSC international FIX inhibitor registry (1997–2006). *Haemophilia* 2009; **15**: 1027–31.

5 High KA. Factor IX: Molecular structure, epitopes, and mutations associated with inhibitor formation. *Adv Exp Med Biol* 1995; **386**: 79–86.

6 Ljung R, Petrini P, Tengborn L, *et al*. Haemophilia B mutations in Sweden: A population-based study of mutational heterogeneity. *Br J Haematol* 2001; **113**: 81–6.

7 Belvini D, Salviato R, Radossi P, *et al*. Molecular genotyping of the Italian cohort of patients with hemophilia B. *Haematologica* 2005; **90**: 635–42.

8 Astermark J, Berntorp E, White GC, *et al*. The Malmo International Brother Study (MIBS): Further support for genetic predisposition to inhibitor development in hemophilia patients. *Haemophilia* 2001; **7**: 267–72.

9 Green P. A database of point mutations and short additions and deletions in the factor IX gene. 2004. Available at: www.kcl.ac.uk/ip/petergreen/haemBdatabase.html.

10 Christophe OD, Lenting PJ, Cherel G, *et al*. Functional mapping of anti-factor IX inhibitors developed in patients with severe hemophilia B. *Blood* 2001; **98**: 1416–23.

11 Carroll RR, Panush RS, Kitchens CS. Spontaneous disappearance of an IgA anti-factor IX inhibitor in a child with Christmas disease. *Am J Hematol* 1984; **17**: 321–5.

12 Dioun AF, Ewenstein BM, Geha RS, *et al*. IgE-mediated allergy and desensitization to factor IX in hemophilia B. *J Allergy Clin Immunol* 1998; **102**: 113–17.

13 Curry NS, Misbah SA, Giangrande PL, *et al*. Achievement of immune tolerance in a patient with haemophilia B and inhibitory antibodies, complicated by an anaphylactoid reaction. *Haemophilia* 2007; **13**: 328–30.

14 Luu H, Ewenstein B. FEIBA safety profile in multiple modes of clinical and home-therapy application. *Haemophilia* 2004; **10**(Suppl. 2): 10–16.

15 Warrier I. Management of haemophilia B patients with inhibitors and anaphylaxis. *Haemophilia* 1998; **4**: 574–6.

16 Teitel JM, Barnard D, Israels S, *et al*. Home management of haemophilia. *Haemophilia* 2004; **10**: 118–33.

17 Key NS, Aledort LM, Beardsley D, *et al*. Home treatment of mild to moderate bleeding episodes using recombinant factor VIIa (Novoseven) in haemophiliacs with inhibitors. *Thromb Haemost* 1998; **80**: 912–18.

18 Lusher JM, Roberts HR, Davignon G, *et al*. A randomized, double-blind comparison of two dosage levels of recombinant factor VIIa in the treatment of joint, muscle and mucocutaneous haemorrhages in persons with haemophilia A and B, with and without inhibitors. rFVIIa Study Group. *Haemophilia* 1998; **4**: 790–8.

19 Dimichele D, Negrier C. A retrospective postlicensure survey of FEIBA efficacy and safety. *Haemophilia* 2006; **12**: 352–62.

20 Kavakli K, Makris M, Zulfikar B, *et al*. Home treatment of haemarthroses using a single dose regimen of recombinant activated factor VII in patients with haemophilia and inhibitors. A multicentre, randomised, double-blind, cross-over trial. *Thromb Haemost* 2006; **95**: 600–5.

21 Stine KC, Shrum D, Becton DL. Use of FEIBA for invasive or surgical procedures in patients with severe hemophilia A or B with inhibitors. *J Pediatr Hematol Oncol* 2007; **29**: 216–21.

22 Lusher JM. Thrombogenicity associated with factor IX complex concentrates. *Semin Hematol* 1991; **28**(Suppl. 6): 3–5.

23 Abshire T, Kenet G. Safety update on the use of recombinant factor VIIa and the treatment of congenital and acquired deficiency of factor VIII or IX with inhibitors. *Haemophilia* 2008; **14**: 898–902.

24 O'Connell KA, Wood JJ, Wise RP, *et al*. Thromboembolic adverse events after use of recombinant human coagulation factor VIIa. *JAMA* 2006; **295**: 293–8.

25 Young G, Shafer FE, Rojas P, *et al*. Single 270 microg kg(–1)-dose rFVIIa vs. standard 90 microg kg(–1)-dose rFVIIa and APCC for home treatment of joint bleeds in haemophilia patients with inhibitors: A randomized comparison. *Haemophilia* 2008; **14**: 287–94.

26 Ng HJ, Lee LH. Recombinant activated clotting factor VII (rFVIIa) in the treatment of surgical and spontaneous bleeding episodes in hemophilic patients. *Vasc Health Risk Manag* 2006; **2**: 433–40.

27 Kenet G, Martinowitz U. Single-dose recombinant activated factor VII therapy in hemophilia patients with inhibitors. *Semin Hematol* 2008; **45**(Suppl. 1): S38–41.

28 Shapiro AD. Single-dose recombinant activated factor VII for the treatment of joint bleeds in hemophilia patients with inhibitors. *Clin Adv Hematol Oncol* 2008; **6**: 579–86.

29 Lusher JM. Early treatment with recombinant factor VIIa results in greater efficacy with less product. *Eur J Haematol* 1998; **63**(Suppl.): 7–10.

30 Schneiderman J, Rubin E, Nugent DJ, *et al*. Sequential therapy with activated prothrombin complex concentrates and recombinant FVIIa in patients with severe haemophilia and inhibitors: Update of our previous experience. *Haemophilia* 2007; **13**: 244–8.

31 Scalone L, Mantovani LG, Manucci PM, *et al*. Quality of life is associated to the orthopaedic status in haemophilic patients with inhibitors. *Haemophilia* 2006; **12**: 154–62.

32 Young G, McDaniel M, Nugent DJ. Prophylactic recombinant factor VIIa in haemophilia patients with inhibitors. *Haemophilia* 2005; **11**: 203–7.

33 Konkle BA, Ebbesen LS, Erhardtsen E, *et al*. Randomized, prospective clinical trial of recombinant factor VIIa for secondary prophylaxis in hemophilia patients with inhibitors. *J Thromb Haemost* 2007; **5**: 1904–13.

34 Leissinger CA, Becton DL, Ewing NP, *et al*. Prophylactic treatment with activated prothrombin complex concentrate (FEIBA) reduces the frequency of bleeding episodes in paediatric patients with haemophilia A and inhibitors. *Haemophilia* 2007; **13**: 249–55.

35 Royal S, Schramm N, Berntorp E, *et al*. Quality-of-life differences between prophylactic and on-demand factor replacement therapy in European haemophilia patients. *Haemophilia* 2002; **8**: 44–50.

36 Brackmann HH. Induced immunotolerance in factor VIII inhibitor patients. *Prog Clin Biol Res* 1984; **150**: 181–95.

37 Nilsson IM, Sundqvist SB. Suppression of secondary antibody response by intravenous immunoglobulin and development of tolerance in a patient with haemophilia B and antibodies. *Scand J Haematol* 1984; **40**(Suppl.): 203–6.

38 Brackmann HH, Oldenburg J, Schwaab R. Immune tolerance for the treatment of factor VIII inhibitors—Twenty years' "bonn protocol". *Vox Sang* 1996; **70**(Suppl. 1): 30–5.

39 Nilsson IM, Jonsson S, Sundqvist SB, *et al*. A procedure for removing high titer antibodies by extracorporeal protein-A-sepharose adsorption in hemophilia: Substitution therapy and surgery in a patient with hemophilia B and antibodies. *Blood* 1981; **58**: 38–44.
40 Freiburghaus C, Berntorp E, Ekman M, *et al*. Immunoadsorption for removal of inhibitors: Update on treatments in Malmo-Lund between 1980 and 1995. *Haemophilia* 1998; **4**: 16–20.
41 Freiburghaus C, Berntorp E, Ekman M, *et al*. Tolerance induction using the Malmo treatment model 1982–1995. *Haemophilia* 1999; **5**: 32–9.
42 DiMichele DM, Kroner BL. Analysis of the North American Immune Tolerance Registry (NAITR) 1993–1997: Current practice implications. ISTH Factor VIII/IX Subcommittee Members. *Vox Sang* 1999; **77**(Suppl. 1): 31–2.
43 Berntorp E, Astermark J, Carlborg E. Immune tolerance induction and the treatment of hemophilia. Malmo protocol update. *Haematologica* 2000; **85**(Suppl.): 48–50; discussion 50–1.
44 Alexander S, Hopewell S, Hunter S, *et al*. Rituximab and desensitization for a patient with severe factor IX deficiency, inhibitors, and history of anaphylaxis. *J Pediatr Hematol Oncol* 2008; **30**: 93–5.
45 Ewenstein BM, Takemoto C, Warrior I, *et al*. Nephrotic syndrome as a complication of immune tolerance in hemophilia B. *Blood* 1997; **89**: 1115–16.
46 Dharnidharka VR, Takemoto C, Ewenstein BM, *et al*. Membranous glomerulonephritis and nephrosis post factor IX infusions in hemophilia B. *Pediatric Nephrology* 1998; **12**: 654–7.
47 Tengborn L, Hansson S, Fasth A, *et al*. Anaphylactoid reactions and nephrotic syndrome—a considerable risk during factor IX treatment in patients with haemophilia B and inhibitors: A report on the outcome in two brothers. *Haemophilia* 1998; **4**: 854–9.22
48 Cross DC, Van Der Berg HM, Cross DCA. Cyclosporin A can achieve immune tolerance in a patient with severe haemophilia B and refractory inhibitors. *Haemophilia* 2007; **13**: 111–14.
49 Klarmann D, Martinez Saguer I, Funk MB, *et al*. Immune tolerance induction with mycophenolate-mofetil in two children with haemophilia B and inhibitor. *Haemophilia* 2008; **14**: 44–9.
50 Chuansumrit A, Moonsup Y, Sirachainan N, *et al*. The use of rituximab as an adjuvant for immune tolerance therapy in a hemophilia B boy with inhibitor and anaphylaxis to factor IX concentrate. *Blood Coagul Fibrinolysis* 2008; **19**: 208–11.
51 Berman BW, McIntosh S, Clyne LP, *et al*. Spontaneously acquired Factor IX inhibitors in childhood. *Am J Pediatr Hematol Oncol* 1981; **3**: 77–81.
52 Mazzucconi MG, Peraino M, Bizzoni L, *et al*. Acquired inhibitor against factor IX in a child: Successful treatment with high-dose immunoglobulin and dexamethasone. *Haemophilia* 1999; **5**: 132–4.
53 Miller K, Neely JE, Krivit W, *et al*. Spontaneously acquired factor IX inhibitor in a nonhemophiliac child. *J Pediatr* 1978; **93**: 232–4.
54 Ozsoylu S, Ozer FL. Acquired factor IX deficiency. A report of two cases. *Acta Haematol* 1973; **50**: 305–14.
55 Collins HW, Gonzalez MF. Acquired factor IX inhibitor in a patient with adenocarcinoma of the colon. *Acta Haematol* 1984; **71**: 49–52.
56 Lecumberri R, Panzio C, Paramo JA, *et al*. Acquired inhibitor of the intrinsic pathway in a non-haemophilic patient. Control of bleeding by recombinant factor VIIa. *Br J Haematol* 2002; **119**: 284–5.

16 Pharmacokinetics

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Why pharmacokinetics?

Treatment with a drug aims to produce a certain pharmacologic effect for a certain time. This requires that the drug attains an appropriate concentration at its site of action and is then eliminated from this site when the effect is no longer desired. The processes governing drug concentrations are summarized as the pharmacokinetics of the drug. The relationship between drug concentration at the site of action and an observable effect is then the pharmacodynamics of the drug. The pharmacokinetics and pharmacodynamics together determine the necessary dose, dosing intervals, and mode of administration of the drug. If a drug is prescribed according to general guidelines with or without empirical adjustment of the dosing, then the "black box" of pharmacokinetics and pharmacodynamics remains unopened. In many instances this can be entirely justified. However, treatment of hemophilia is eminently suited to optimization by pharmacokinetic methods. The therapeutic plasma levels of factor VIII (FVIII) or factor IX (FIX) in various clinical situations are reasonably known and the methods to achieve, maintain, and monitor these levels are well established. The aim of this chapter is to review the pharmacokinetics of FVIII and FIX and to give an outline of clinical applications to optimize the treatment of hemophilia. It is based on several published review articles [1–4].

Assays and plasma levels

Coagulation factors are precursors of enzymes or cofactors in the coagulation cascade. Plasma concentrations of these proteins are difficult to measure and sometimes irrelevant since molar concentrations may not directly translate into biologic activity. Consequently, their "concentrations" in plasma are normally determined by bioassays and expressed as coagulant activities in international units (U) per milliliter or deciliter. For FVIII and FIX the coagulant activities will be denoted FVIII:C and FIX:C, respectively. Since the word "concentration" is obviously inappropriate to refer to coagulant activity the word "level" will be preferred. By definition, 1 U/mL, 1 kU/L, or 100 U/dL is the average coagulation factor level in healthy individuals.

The methods currently used to measure FVIII:C and FIX:C are the one-stage assay and the chromogenic substrate two-stage assay (see Chapters 38 and 39). Discrepancies between different bioassays may complicate the interpretation of pharmacokinetic data since they affect the determination of both administered dose and plasma levels. The problems are most pronounced for the measurement of the *in vitro* potency of a factor concentrate. The greatest difference between traditional one-stage assays and the chromogenic two-stage assay has been observed for B-domain-deleted recombinant FVIII [5]. These difficulties must be overcome. Guidelines on how to minimize the assay problems, using product-specific standards for FVIII, have been published [6–8].

The advantage of defining plasma levels using bioassays is that these levels as functions of time represent the kinetics of the desired coagulant effect. Pharmacokinetics properly deals with drug concentrations. Applying pharmacokinetic calculations to coagulant activity values is thus questionable in principle; however, it is very convenient in practice.

Methods, definitions, and applications of pharmacokinetics

The following parameters are normally used to characterize the disposition or "pharmacokinetics" of the coagulation factors [1,2]:

- *Clearance (CL).* Clearance is the capacity of the body to eliminate a substance, usually expressed as the volume of plasma that is cleared of substance in 1 min or 1 h. The best-known clearance in the medical literature is creatinine clearance. However, the concept can be applied to any substance, either endogenous or exogenous. The clearance of a drug is normally calculated as dose divided by the area under the plasma concentration curve (AUC). During a constant-rate infusion this corresponds to rate of infusion divided by plasma concentration at steady state.
- *Volume of distribution (V).* This is defined as the amount of drug in the body divided by the plasma concentration. Thus, it represents the apparent volume of plasma in which a drug is distributed (or diluted) in the body. If, for example, 1500 U of FVIII is injected and the plasma level immediately

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afterwards is 0.5 U/mL then the FVIII appears to have been distributed into 3000 mL of plasma. As drugs in general diffuse gradually from the circulation into various organs and tissues, *V* normally increases over time after the injection. At distribution equilibrium, *V* is the volume of distribution at steady state (V_{dss}). The higher the V_{dss}, the more extensive is the distribution of the drug away from the plasma space.

- *Mean residence time (MRT).* This represents the average lifetime of the drug molecules in the body. The MRT depends on both distribution and elimination and can be calculated very simply as V_{dss} divided by clearance.
- *Half-life ($t_{½}$).* The processes of distribution and elimination also govern the half-life of a drug. However, the plasma concentration curve of a drug normally shows several half-lives (or phases)—at least one early phase owing to distribution and the "terminal" phase representing elimination. Half-lives are determined by fitting an exponential equation to the plasma concentration versus time values (or in simple cases a linear function to log plasma concentration versus time values, which is equivalent). This curve-fitting is illustrated in Figures 16.1 and 16.2.
- *In vivo recovery (IVR).* This parameter has only been used for coagulation factors. IVR is calculated as observed peak FVIII:C or FIX:C divided by the expected peak activity (or simply as peak level divided by dose). The expected activity is the dose divided by the plasma volume of the patient. As will be described below, the assumption that the initial *V* equals the plasma volume may be approximately valid for FVIII:C but not for FIX:C [1,2,9]. A second problem is that the plasma volume of a subject is seldom accurately known. It is, for instance, not linearly related to total body weight [10]. A third problem is that of a "postinfusion activation" (see below for FVIII) that makes the IVR dependent on the early blood sampling schedule in the pharmacokinetic study [1,2,11,12].

CL and *V* (V_{dss}) are often normalized to the body weight of the patient and expressed as, for example, mL/h/kg and L/kg. However, CL and *V* are seldom directly proportional to body weight; thus, this normalization may not always diminish interindividual variance.

The pharmacokinetic parameters as such are useful to describe the *in vivo* behavior of FVIII and FIX and to compare preparations of coagulation factors [1,2,11,12]. Of greater clinical interest, however, is that they can be used to calculate plasma levels of FVIII:C or FIX:C at any time during a treatment [13–15]. This will be illustrated below. Most commonly trough (minimum) and peak levels, i.e., levels found immediately before and after administration of a dose, during ongoing treatment, are discussed.

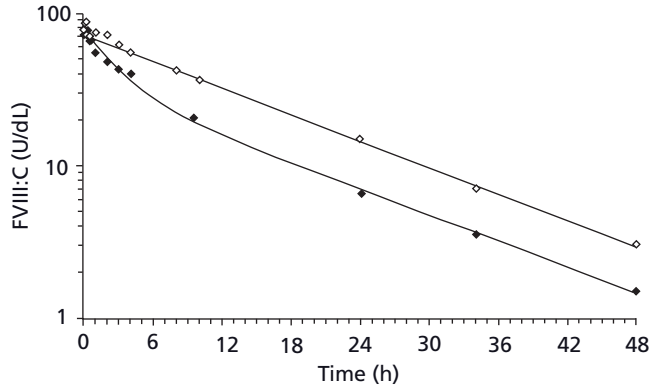

Figure 16.1 Representative FVIII:C versus time curves after intravenous administration of FVIII to adult patients with severe hemophilia A [12]. The upper curve (open symbols) is practically monophasic. After some slight early irregularities, it declines with a single half-life of 10 h. The exponential equation describing the curve is $C(t) = 72 \times e^{-0.067 \times t}$, where $C(t)$ is the "concentration" of FVIII:C as a function of time and t is time. The lower curve (closed symbols) is obviously biphasic. The two half-lives are 1.9 and 10 h and the exponential equation is $C(t) = 45 \times e^{-0.36 \times t} + 34 \times e^{-0.066 \times t}$. The early phase contributes 20% of the area under the curve.

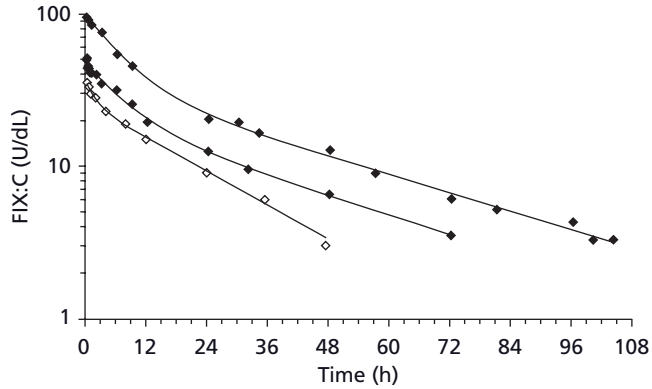

Figure 16.2 Representative FIX:C versus time curves after intravenous administration of FIX to adult patients with severe hemophilia B. They are taken from three different studies. Uppermost curve: plasma-derived FIX was given in a dose of 80 U/kg [9]. The curve is described by the function $C(t) = 63 \times e^{-0.15 \times t} + 35 \times e^{-0.023 \times t}$. The half-lives of the phases are 4.7 and 30 h. Middle curve: plasma-derived FIX was given in a dose of 50 U/kg [39]. The curve is described by the function $C(t) = 26 \times e^{-0.13 \times t} + 20 \times e^{-0.024 \times t}$. The half-lives of the phases are 5.2 and 28 h. Lower curve (open symbols): recombinant FIX was given in a dose of 50 U/kg [40,41]. The curve is described by the function $C(t) = 10 \times e^{-0.51 \times t} + 26 \times e^{-0.043 \times t}$. The half-lives of the phases are 1.4 and 16 h. Lower levels of FIX:C were obtained after administration of recombinant as compared with plasma-derived FIX in spite of the dose being the same. This is reflected by the clearance values calculated from the three curves: plasma-derived FIX 4.0 and 4.9 mL/h/kg, respectively, and recombinant FIX 8.0 mL/h/kg.

Pharmacokinetics of factor VIII

In healthy persons, FVIII is presumably produced mainly in the liver and circulates in the plasma bound to the von Willebrand factor (VWF) [16]. In patients with hemophilia A this complex is rapidly formed between infused FVIII and endogenous VWF. The binding of FVIII to VWF protects FVIII from degradation and receptor-mediated clearance [17–19]. Thus, FVIII infused as a highly purified or recombinant

concentrate is cleared much faster in patients with severe von Willebrand disease, who lack functional VWF, than in patients with hemophilia A [20,21]. The very high molecular weight of the FVIII–VWF complex practically confines it to the plasma space. It can be calculated [2] that only about 14% of the body load of FVIII is extravascular at steady state. Receptor-mediated cellular uptake of the FVIII–VWF complex is probably an important mechanism of FVIII clearance [19].

When FVIII is given to adult patients as short-term infusions (typically of 5–15 min duration), plasma FVIII:C levels, on average, rise by 0.020–0.025 U/mL for every U/kg administered. Thus, an infusion of 50 U/kg will normally give a peak plasma level of 1.0–1.3 U/mL. This corresponds to an initial V of 0.04–0.05 L/kg. The plasma disposition curve of FVIII:C is then often approximately monophasic (Figure 16.1). Even if an irregular early phase can often be discerned it seldom contributes much to the total AUC. This early phase may represent more than one process. One would be some (very limited) distribution of FVIII to intra- or extravascular sites, and the other process could be rapid clearance of high-molecular-weight forms and aggregates of FVIII by the reticuloendothelial system. In addition, the peak plasma FVIII:C is often found 10–15 min after the end of the FVIII infusion, or sometimes even 1–2 h later [1,2,4,11,12]. The reason for this postinfusion rise in activity is not known.

The pharmacokinetics of FVIII:C in patients with hemophilia are summarized in Table 16.1. The parameter values apply under normal physiologic conditions and in the absence of inhibitors (antibodies) to FVIII. The presence of inhibitors may give a low IVR and/or a rapid clearance of FVIII:C [2,11,22]. The differences between plasma-derived and the presently available types of recombinant FVIII are marginal, even though some of them were statistically significant in comparative crossover studies.

There is marked interindividual variation in the pharmacokinetics of FVIII:C. In adult patients, the CL of plasma-derived FVIII:C typically varies between 1.8 and 6 mL/h/kg and $t_{½}$ between 8 and 23 h [1,2,12,23]. Some of the variance can be explained by physiologic factors. Since FVIII is highly bound to VWF, variability in the plasma concentration and/or CL of endogenous VWF likely explains one source of variability in CL and $t_{½}$ of FVII:C. It was accordingly found that the CL of B-domain-deleted recombinant FVIII correlates negatively, and the $t_{½}$ positively, with VWF levels in patients with hemophilia A [24]. This has been observed also with plasma-derived and full-length recombinant FVIII [25,26]. Blood group may also play a role. In one study, FVIII:C had a significantly shorter mean elimination $t_{½}$ in patients with group O than in patients with group A [27]. The influences of blood group and VWF level are inter-related, since group O individuals also on average have lower levels of VWF [25,28].

The relationship of age of the patient with the pharmacokinetics of FVIII has been studied in subjects from 1 year of age and upwards [14,25,26,29,30]. Chronological age is a substitute parameter for the changes in body weight and composition as well as in the efficacy of clearance mechanisms that take place during growth and that actually affect the pharmacokinetics of FVIII. Growing into adulthood may increase the body proportion of fat, which plays no part in the distribution and elimination of a coagulation factor. In addition, baseline VWF level increases with age [25,28]. It was thus observed in 41 patients aged 9–70 years that the weight-adjusted CL of FVIII:C (i.e., in mL/h/kg) decreased with the age of the patients [29]. In a study on 53 children aged 1–6 years [30] the mean CL of recombinant FVIII was 4.4 mL/h/kg, i.e., markedly higher than in adults (cf. Table 16.1). Pooled findings from several studies [2,14,25,26,30] indicate that the average terminal $t_{½}$ of FVIII:C increases with age, from approximately 9 h at 1 year of age to around 14 h in the mature adult. Interindividual variation is large, however.

Body weight and composition influence FVIII pharmacokinetics both as correlates with age and by themselves. Doubling of the body weight (from 40 to 80 kg) in patients aged between 8 and 42 years was associated with a 42% increase in CL and a 60% increase in V_{dss} [14], i.e., the changes were less than proportional to weight. IVR, when calculated assuming that plasma volume is a constant proportion of total body weight, tends to increase with the weight of the patient [12,31,32]. This is likely explained by the fact that plasma volume as a fraction of total body weight decreases with weight [10].

Crossover studies on different FVIII concentrates indicate that the disposition of FVIII:C remains fairly constant within an individual, i.e. intraindividual variation in pharmacokinetics is lower than the interindividual variation [12,33–35].

Table 16.1 Reported mean pharmacokinetic parameter values of factor VIII (FVIII), compiled from studies on plasma-derived FVIII concentrates (pdFVIII, range of mean values from six studies), full-length recombinant FVIII (rFVIII, one study) and B-domain-deleted FVIII (rFVIIISQ, one study). Data are taken from a published review [2].

	CL (mL/h/kg)	V_{dss} (L/kg)	MRT (h)	Terminal $t_{½}$ (h)
pdFVIII	2.4–3.4	0.04–0.06	14–21	11–15
rFVIII	2.5	0.05	21	16
rFVIII SQ	3.2	0.05	16	11

Pharmacokinetics of factor IX

Factor IX is produced by the liver and circulates in the plasma as a free molecule. Owing to its low molecular weight (55 kDa), it also readily diffuses into the interstitial fluid [36]. FIX also binds rapidly and reversibly to the vascular endothelium, with a half-maximal binding concentration comparable to its normal concentration in plasma [37,38]. There are conflicting

Table 16.2 Reported mean pharmacokinetic parameter values of factor IX (FIX), compiled from studies with plasma-derived FIX concentrates (pdFIX, range of mean values from five studies) or recombinant FIX (rFIX, two studies; CL and V_{dss} are given in only one of them). Data are taken from a published review [2].

	CL (mL/h/kg)	V_{dss} (L/kg)	MRT (h)	Terminal $t_{½}$ (h)
pdFIX	3.8–4.3	0.11–0.15	34–45	29–34
rFIX	8.4	0.22	25–26	18–20

data in the literature on the pharmacokinetics of FIX:C. One reason for this is that many studies have been performed with inadequate blood-sampling protocols, yielding biased or imprecise results [1,9]. A second reason is the difference in pharmacokinetics between plasma-derived and recombinant FIX (see below).

When plasma-derived FIX is given to adult patients as short-term infusions (typically of 5–15 min duration), plasma FIX:C levels on average rise by 0.010–0.014 U/mL for every U/kg administered. Thus, an infusion of 50 U/kg will normally give a peak plasma level of 0.5–0.7 U/mL. This corresponds to an initial V of 0.07–0.10 L/kg, which exceeds the plasma volume. Binding to the endothelium causes immediate disappearance of some of the infused FIX from the plasma. FIX:C then declines in a clearly biexponential fashion (Figure 16.2), in which the distribution phase of the curve represents diffusion of FIX into interstitial fluid. Thus, the V_{dss} of FIX is three- to fourfold greater than the plasma volume.

Table 16.2 summarizes methodologically adequate single-dose pharmacokinetic studies on FIX:C in patients with hemophilia. It can be seen that the terminal $t_{½}$ of FIX:C is longer than that of FVIII:C. The presently available recombinant FIX differs both in biochemistry and pharmacokinetics from plasma-derived FIX, with a higher CL. Its IVR is approximately two-thirds that of plasma-derived FIX [40].

Interindividual variance in the pharmacokinetics of plasma-derived FIX:C is difficult to estimate since the applicable studies include only very few patients. No correlates or causes of interindividual variation in the standard pharmacokinetic parameters have, as yet, been identified. In a large study on recombinant FIX administered to 55 patients aged 4–56 years [40,41], both CL (in mL/h) and V_{dss} (in L) were linearly correlated (but not directly proportional) to body weight, consequently increasing during childhood and adolescence but remaining fairly constant during adulthood. Owing to the similar rises in CL and V_{dss}, neither MRT (= V_{dss}/CL) nor $t_{½}$ showed any significant regression with either body weight or age.

Application of pharmacokinetics to treatment of hemophilia

Clinical pharmacokinetics is the application of pharmacokinetic principles to the therapeutic management of patients [42]. Adjusting the dosage of a drug according to the requirement of the individual patient, which in turn is based on knowledge of the pharmacokinetics of the drug in that particular patient, is often referred to as "tailoring" the dose. Target plasma levels of FVIII:C or FIX:C, as well as the need for dose tailoring, are very different between different clinical situations. These will therefore be dealt with separately. Common to all situations, however, is the fact that coagulation factor concentrates are a precious resource, expensive, and/or of limited availability, which should not be wasted by inappropriate dosing.

Treatment of bleedings and prophylaxis and treatment during and after surgery

Guidelines for therapy and optimal dosing for bleeding in hemophilia have been developed on behalf of the World Federation of Hemophilia (WFH) [43]. The therapeutic FVIII:C or FIX:C level (in this case the peak level after the first dose) recommended for most bleedings, such as joint, muscle, and retroperitoneal bleedings or hematuria, is 0.3–1.0 U/mL. The treatment should be continued for 1–5 days, depending on the severity of the bleeding. For intracranial bleeding the initial peak level should be 0.8–1.0 U/mL and the treatment period should be 8–21 days. In countries with significant resource constraints, lower plasma levels and shorter treatment duration have to be accepted.

The doses of FVIII and FIX can be calculated from the IVR data given above. Thus, for FVIII, the dose (in U/kg) = 50 × (required rise in U/mL FVIII:C), and, for FIX, the dose (U/kg) = 100 × (required rise in U/mL FIX:C). A recommended dosage for an adult patient with an ordinary bleeding would thus be 15–50 U/kg for FVIII and 30–100 U/kg for FIX, with repeat dosing as required every 12–24 h (however, a single dose of at least 30 U/kg FVIII is often sufficient for joint bleedings).

Guidelines for dosing in surgery have also been developed and endorsed by the WFH [43]. For major surgery the preoperative peak factor level should be 0.8–1.0 U/mL for FVIII:C and 0.6–0.8 U/mL for FIX:C. This peak level applies during days 1–3 and is then tapered off until day 12. The dosing interval for the first 4–6 days is 8–12 h for FVIII and 12–18 h for FIX. For minor surgery peak levels can be lower by 40–50% and the total treatment duration shortened. In certain cases higher levels and increased frequency of administration may be required.

Administration of FVIII or FIX as a continuous infusion is becoming popular since maintaining a steady therapeutic plasma level of coagulation factor in this way requires less factor concentrate than maintaining a therapeutic trough level by intermittent injections [2,44]. With a given dosing interval the saving is greater the shorter the $t_{½}$ of the factor activity (since the shorter the $t_{½}$, the higher the peak levels must be in order to maintain the desired trough level) [45]. Further reading on continuous infusion can be found in Chapter 6.

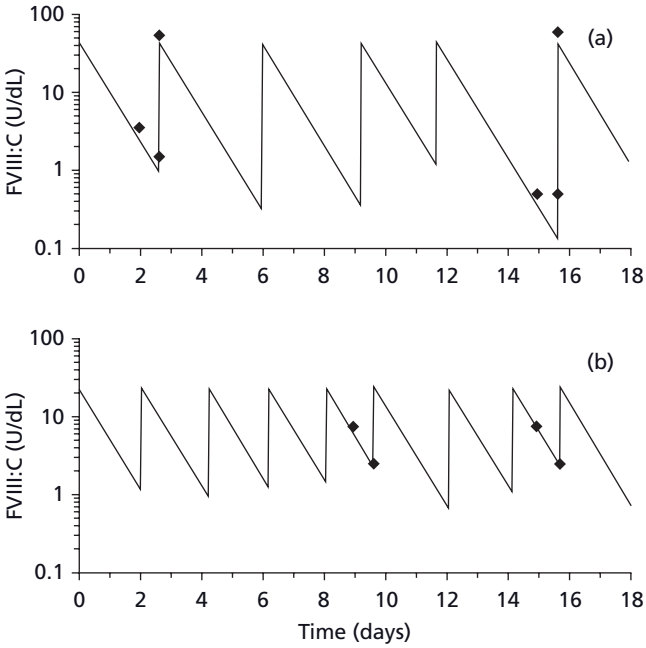

Figure 16.3 Predicted plasma FVIII:C levels in a patient on prophylactic therapy with FVIII [13]. The single-dose curve $C(t) = 56 \times e^{-1.48 \times t}$ (where time, t, is in days) was obtained after a dose of 2300 U [12]. Multiple-dose curves were consequently plotted from the equations $C(t) = (\text{given dose}/2300) \times 56 \times e^{-1.48 \times t}$ which were added over time, each new curve starting when a new dose was given. (a) Originally prescribed dosing of 2000 U twice weekly. (b) Dose tailored to give a trough level of 1 U/dL (0.01 U/mL); 1000 U every two days. Diamonds are measured control values. The two values at 0.5 U/dL are essentially "blank," or baseline, readings in the assay.

Table 16.3 Calculated dose requirements of FVIII, plasma-derived FIX, and recombinant FIX in 70-kg patients to maintain trough plasma levels of 0.01 U/mL during regular prophylactic treatment.

Schedule	Dose needed (U)	Annual consumption (kU/year)[a]
FVIII		
Daily	113 (56; 268)	41 (21; 98)
Every other day	588 (204; 2590)	107 (37; 473)
Every third day	2600 (588; 22800)	316 (72; 2770)
By continuous infusion	50[b] (34; 76)	18 (12; 28)
Plasma-derived factor IX		
Daily	105 (60; 206)	38 (22; 75)
Every other day	297 (148; 790)	54 (27; 144)
Every third day	626 (270; 2260)	76 (33; 274)
By continuous infusion	67[b] (45; 101)	25 (16; 37)
Recombinant factor IX		
Daily	234 (131; 469)	85 (48; 171)
Every other day	794 (365; 2260)	145 (67; 413)
Every third day	2140 (781; 9090)	261 (95; 1110)
By continuous infusion	141[b] (94; 212)	52 (34; 77)

Calculations were performed for average values of pharmacokinetic parameters (FVIII: CL 3.0 mL/h/kg, terminal $t_{½}$ 12 h; plasma-derived FIX: CL 4.0 mL/h/kg, terminal $t_{½}$ 31 h; recombinant FIX: CL 8.4 mL/h/kg, terminal $t_{½}$ 19 h) and also for 1.5-fold lower and higher clearances (values in parentheses). Some doses are obviously unrealistic, in particular administration of coagulation factor every third day to patients in which the CL is higher than average.

[a]kU = 1000 U.

[b]U/day.

The dosage recommendations above include a certain margin of safety and the patient is monitored clinically in most situations. Dose tailoring can be performed by assays of plasma coagulation factor and suitable dose adjustments without elaborate pharmacokinetic calculations.

Prophylactic treatment

Long-term clinical experience and one randomized study indicate that prophylactic treatment which produces trough levels of FVIII:C or FIX:C of 0.01 U/mL is often adequate to prevent bleedings in patients with severe hemophilia [46–48]. The recommended standard doses to achieve this are 25–40 U/kg FVIII three times weekly in hemophilia A and 25–40 U/kg FIX twice weekly in hemophilia B. It is, however, clear that the dosage requirement of FVIII or FIX for prophylactic treatment varies considerably between individuals. There are some patients with biochemically severe disease (i.e., a factor level of <0.01 U/mL) who suffer few bleedings, maintain normal joint function, and sometimes do not require prophylactic treatment. On the other hand, patients with severely damaged joints may require higher trough levels of FVIII:C or FIX:C [3,14,15,46,47]. Dosing during prophylactic treatment must therefore be adjusted according to clinical outcome in terms of bleeding frequency or change in joint status. In spite of this, pharmacokinetics can be regarded as a valuable tool to optimize dosing of FVIII and FIX.

An example of dose tailoring in a patient with hemophilia A is shown in Figure 16.3. A 0.01 U/mL trough level was aimed for and attained. Concomitantly, the consumption of FVIII was lowered from 4000 to 3500 U per week. Several studies [13–15,41] have shown that the cost-effectiveness of prophylactic treatment can be improved considerably in this way. The raised trough levels in conjunction with (normally) decreased consumption of factor concentrate during optimized dosing are chiefly a result of shorter injection intervals and lower doses at each injection, which result in lower peak levels. There is no indication that lower peak levels would result in less effective prophylactic treatment. After all, the original idea of prophylactic treatment [46,47] was to mimic the situation in moderate hemophilia, i.e., a constant coagulation factor level of 0.01–0.05 U/mL with no peaks at all.

Table 16.3 shows calculated annual consumption, with various dose intervals, of FVIII, plasma-derived FIX, or

recombinant FIX in hypothetical "average" adult patients (70 kg, pharmacokinetic parameters as in Tables 16.1 and 16.2) as well as in patients with extreme values of CL and $t_{½}$. In practice, the dosing would have to be adjusted to available vial sizes. The sharp increase in dose requirement with prolongation of the dose interval should be noted. For example, if the $t_{½}$ of the coagulation factor is 12 h then the level will decrease by three-quarters during 24 h. Shifting the single-dose curve upwards from 0.01 U/mL at 24 h to 0.01 U/mL at 48 h thus requires a quadrupling of the dose (and to 0.01 U/mL at 72 h another quadrupling). During repeated dosing the degree of accumulation (i.e., how much the previously given doses will contribute to the total FVIII:C or FIX:C level) will also influence these dose ratios. Long-term prophylaxis by continuous infusion would be very economical as regards factor consumption but is not technically feasible at the time of writing. The choice between injection schedules must balance practical and ethical issues against cost and availability of factor concentrates.

Conclusion

The pharmacokinetics of FVIII and FIX has been extensively investigated and their therapeutic plasma levels are well defined in most situations. Applied pharmacokinetics has therefore become an established tool for dosing in the treatment of hemophilia.

References

1 Björkman S, Carlsson M. The pharmacokinetics of factor VIII and factor IX: Methodology, pitfalls and applications. *Haemophilia* 1997; **3**: 1–8.

2 Björkman S, Berntorp E. Pharmacokinetics of coagulation factors. Clinical relevance for patients with haemophilia. *Clin Pharmacokin* 2001; **40**: 815–32.

3 Björkman S. Prophylactic dosing of factor VIII and factor IX from a clinical pharmacokinetic perspective. *Haemophilia* 2003; **9**(Suppl. 1): 101–10.

4 Berntorp E, Björkman S. The pharmacokinetics of clotting factor therapy. *Haemophilia* 2003; **9**: 353–9.

5 Mikaelsson M, Oswaldsson U, Sandberg H. Influence of phospholipids on the assessment of factor VIII activity. *Haemophilia* 1998; **4**: 646–50.

6 Barrowcliffe TW. Recommendations for the assay of high-purity factor VIII concentrates. *Thromb Haemost* 1993; **70**: 876–7.

7 Mikaelsson M, Oswaldsson U. Assaying the circulating factor VIII activity in hemophilia A patients treated with recombinant factor VIII products. *Sem Thromb Hemost* 2002; **28**: 257–64.

8 Ingerslev J, Jankowski MA, Weston SB, Charles LA, and the Refacto field study participants. Collaborative field study on the utility of a BDD factor VIII concentrate standard in the estimation of BDDr Factor VIII:C activity in hemophilic plasma using one-stage clotting assays. *J Thromb Haemost* 2004; **2**: 623–8.

9 Björkman S, Carlsson M, Berntorp E. Pharmacokinetics of factor IX in patients with haemophilia B: Methodological aspects and physiological interpretation. *Eur J Clin Pharmacol* 1994; **46**: 325–32.

10 Feldschuh J, Enson Y. Prediction of the normal blood volume. Relation of blood volume to body habitus. *Circulation* 1977; **56**: 605–12.

11 Allain JP. Principles of in vivo recovery and survival studies. *Scand J Haematol* 1984; **33**(Suppl. 41): 123–30.

12 Björkman S, Carlsson M, Berntorp E, Stenberg P. Pharmacokinetics of factor VIII in humans: Obtaining clinically relevant data from comparative studies. *Clin Pharmacokin* 1992; **22**: 385–95.

13 Carlsson M, Berntorp E, Björkman S, Lindvall K. Pharmacokinetic dosing in prophylactic treatment of hemophilia A. *Eur J Haematol* 1993; **51**: 247–52.

14 Carlsson M, Berntorp E, Björkman S, Lethagen S, Ljung R. Improved cost-effectiveness by pharmacokinetic dosing of factor VIII in prophylactic treatment of haemophilia A. *Haemophilia* 1997; **3**: 96–101.

15 Carlsson M, Björkman S, Berntorp E. Multidose pharmacokinetics of factor IX: Implications for dosing in prophylaxis. *Haemophilia* 1998; **4**: 83–8.

16 Lenting P, van Mourik JA, Mertens K. The life cycle of coagulation factor VIII in view of its structure and function. *J Am Soc Hematol* 1998; **92**: 3983–96.

17 Weiss HJ, Sussman II, Hoyer LW. Stabilization of factor VIII in plasma by the von Willebrand factor. *J Clin Invest* 1977; **60**: 390–404.

18 Noe DA. A mathematical model of coagulation factor VIII kinetics. *Hemostasis* 1996; **26**: 289–303.

19 Lenting PJ, van Schooten CJM, Denis CV. Clearance mechanisms of von Willebrand factor and factor VIII. *J Thromb Haemost* 2007; **5**: 1353–60.

20 Tuddenham EGD, Lane RS, Rotblat F, Johnson AJ, Snape TJ, Middleton S, Kernoff PBA. Response to infusions of polyelectrolyte fractionated human factor VIII concentrate in human haemophilia A and von Willebrand's disease. *Br J Haematol* 1982; **52**: 259–67.

21 Lethagen S, Berntorp E, Nilsson IM. Pharmacokinetics and hemostatic effect of different factor VIII/von Willebrand factor concentrates in von Willebrand's disease type III. *Ann Hematol* 1992; **65**: 253–9.

22 Allain JP, Frommel D. Antibodies to factor VIII. V. Patterns of immune response to factor VIII in hemophilia A. *Blood* 1976; **47**: 973–82.

23 Smith KJ, Lusher JM, Cohen AR, Salzman P. Initial clinical experience with a new pasteurized monoclonal antibody purified factor VIIIC. *Semin Hematol* 1990; **27**(Suppl. 2): 25–9.

24 Fijnvandraat K, Peters M, Ten Cate JW. Inter-individual variation in half-life of infused recombinant factor VIII is related to pre-infusion von Willebrand factor antigen levels. *Br J Haematol* 1995; **91**: 474–6.

25 Van Dijk K, van der Bom JG, Lenting PJ, *et al*. Factor VIII half-life and clinical phenotype of severe hemophilia A. *Haematologica* 2005; **90**: 494–8.

26 Barnes C, Lillicrap D, Pazmino-Canizares J, *et al*. Pharmacokinetics of recombinant factor VIII (Kogenate-FS) in children and causes of inter-patient pharmacokinetic variability. *Haemophilia* 2006; **12**(Suppl. 4): 40–9.

27 Vlot AJ, Mauser-Bunschoten EP, Zarkova AG, *et al*. The half-life of infused factor VIII is shorter in hemophiliac patients with blood group 0 than in those with blood group A. *Thromb Haemostas* 2000; **83**: 65–9.

28 O'Donnell J, Laffan MA. The relationship between ABO histo-blood group, factor VIII and von Willebrand factor. *Transfus Med* 2001; **11**: 343–51.
29 Matucci M, Messori A, Donati-Cori G, *et al*. Kinetic evaluation of four factor VIII concentrates by model-independent methods. *Scand J Haematol* 1985; **34**: 22–8.
30 Blanchette VS, Shapiro AD, Liesner RJ, *et al*. Plasma and albumin-free recombinant factor VIII: Pharmacokinetics, efficacy and safety in previously treated pediatric patients. *J Thromb Haemost* 2008; **6**: 1319–26.
31 Rousell RH, Kasper CK, Schwartz RS. The pharmacology of a new pasteurized antihemophilic factor concentrate derived from human blood plasma. *Transfusion* 1989; **29**: 208–12.
32 Aronstam A, McLellan DS, Wassef M, Mbatha PS. Effect of height and weight on the in vivo recovery of transfused factor VIII C. *J Clin Pathol* 1982; **35**: 289–91.
33 Nilsson IM, Berntorp E. Clinical efficacy of clotting factor concentrates: Survival, recovery and haemostatic capacity. In: Smit Sibinga C Th, Das PC, Mannucci PM (eds.) *Coagulation and Blood Transfusion* (Proceedings of the Fifteenth Annual Symposium for Blood Transfusion, Groningen 1990). Dordrecht: Kluwer Academic Publishers, **1991**: 193–206.
34 Fijnvandraat K, Berntorp E, ten Cate JW, *et al*. Recombinant, B-domain deleted factor VIII (r-VIII SQ): Pharmacokinetics and initial safety aspects in hemophilia A patients. *Thromb Haemost* 1997; **77**: 298–302.
35 Tarantino MD, Collins PW, Hay CRM, *et al*. Clinical evaluation of an advanced category antihaemophilic factor prepared using a plasma/albumin-free method: Pharmacokinetics, efficacy, and safety in previously treated patients with haemophilia A. *Haemophilia* 2004; **10**: 428–37.
36 Thompson AR. Factor IX concentrates for clinical use. *Semin Thromb Haemostas* 1993; **19**: 25–36.
37 Heimark RL, Schwartz SM. Binding of coagulation factors IX and X to the endothelial cell surface. *Biochem Biophys Res Commun* 1983; **111**: 723–31.
38 Stern DM, Drillings M, Nossel HL, Hurlet-Jensen A, LaGamma KS, Owen J. Binding of factors IX and IXa to cultured vascular endothelial cells. *Proc Nat Acad Sci USA* 1983; **80**: 4119–23.
39 Berntorp E, Björkman S, Carlsson M, Lethagen S, Nilsson IM. Biochemical and in vivo properties of high purity factor IX concentrates. *Thromb Haemostas* 1993; **70**: 768–73.
40 White G, Shapiro A, Ragni M, Garzone P, Goodfellow J, Tubridy K, Courter S. Clinical evaluation of recombinant factor IX. *Semin Hematol* 1998; **35**(Suppl. 2): 33–8.
41 Björkman S, Shapiro AD, Berntorp E. Pharmacokinetics of recombinant factor IX in relation to age of the patient: Implications for dosing in prophylaxis. *Haemophilia* 2001; **7**: 133–9.
42 Rowland M, Tozer TN. *Clinical Pharmacokinetics: Concepts and Applications*, 3rd edn. Baltimore: Williams & Wilkins, 1995.
43 Guidelines for the management of hemophilia. World Federation of Hemophilia, 2005. Available at: www.wfh.org.
44 Schulman S. Continuous infusion. *Haemophilia* 2003; **9**: 368–75.
45 Hay CRM, Doughty HI, Savidge GF. Continuous infusion of factor VIII for surgery and major bleeding. *Blood Coagul Fibrinolysis* 1996; **7**(Suppl. 1): S15–9.
46 Nilsson IM, Berntorp E, Löfqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. *J Intern Med* 1992; **232**: 25–32.
47 Löfqvist T, Nilsson IM, Berntorp E, Pettersson H. Haemophilia prophylaxis in young patients—a long-term follow-up. *J Intern Med* 1997; **241**: 395–400.
48 Manco-Johnson MJ, Abshire TC, Shapiro AD, *et al*. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; **357**: 535–44.

17 The neonate with hemophilia

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Introduction

The neonatal period spans the first 28 days of life of a live-born infant of any gestation. Although acquired disorders of coagulation are more frequent during this period, severe forms of congenital factor deficiencies such as hemophilia A and B often present in the early neonatal period and are a particular challenge from both the diagnostic and management point of view. There should be a high index of suspicion of such disorders in an otherwise healthy infant who presents with unusual bleeding. The proportion of children with hemophilia who are diagnosed in the neonatal period has improved significantly over the past 40 years. Baehner and Strauss reported in 1966 that fewer than 10% of severe hemophiliacs were identified in the newborn period despite unusual bleeding in 22% [1]. Subsequent studies have shown that between 52% and 68% of babies with severe hemophilia are diagnosed as neonates [2–4]. However, despite a positive family history, up to 59% are only diagnosed at the time of the first bleed [4] and the diagnosis is delayed despite unusual bleeding in up to 87.5% [1,4,5]. The normal baby is adapted for the trauma of birth. Levels of coagulation proteins, their inhibitors, and fibrinolytic proteins are age specific and at birth are physiologically balanced to help protect against the risk of bleeding with such trauma. However, in a baby with hemophilia, the balance is altered and the risk of bleeding during birth is significantly increased. Birth is one of the most critical times for intracerebral bleeding in patients with hemophilia [6] and can be the first presenting sign of the disease. In around 50% of individuals with hemophilia there is no family history at birth and therefore preventative measures cannot be instituted. However, where the fetus is known to have, or be at risk of having, hemophilia, it is important to take proactive steps to reduce birth trauma. This is usually advised for male infants but care should also be taken with female infants of known carriers as their factor levels may be significantly reduced and predispose to bleeding in certain circumstances.

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Family history and genetics of hemophilia

Hemophilia A and B are inherited as X-linked recessive bleeding disorders. A number of cohort studies have examined the initial presentation of these conditions and have found that in 42–57% of cases there is no apparent prior family history of hemophilia [1–4,7]. It is estimated from molecular studies that at least 30% of newly diagnosed cases of hemophilia occur as a consequence of a new mutation, affecting either the male propositus or a female carrier in whom there may be no personal history of bleeding problems [8]. It is also evident from published studies that even where there is a positive family history of hemophilia, the history is not always recognized and a proportion of such cases are only diagnosed after they present with clinically overt bleeding symptoms [3].

Hemostatic challenges in the neonatal period

Precipitating factors for bleeding specific to the neonatal period are mostly associated with birth trauma [6,9]. One study showed that in nearly 600 000 unselected infants of nulliparous mothers, the rate of intracranial hemorrhage (ICH) was higher among infants delivered by vacuum extraction, forceps, or cesarean section during labor than infants delivered spontaneously or by elective cesarean section [10]. A prospective study from Whitby and colleagues showed that subdural hemorrhage can occur with normal vaginal delivery but was more frequent in those with instrumental deliveries [11]. This suggests that the common risk factor for hemorrhage is abnormal labor. Although no information was given regarding outcome or coagulation status of these infants, it would seem reasonable to extrapolate these results and assume that these types of birth are more risky for babies with hemophilia. This has been supported by several studies [6,9] where instrumentation, particularly vacuum extraction, has been associated with an increase in both ICH and extracranial hemorrhage (ECH) (Figures 17.1 and 17.2). A study by Tarantino and colleagues reviewed data on 1×10^7 neonates and identified 580 with hemophilia or von Willebrand disease.

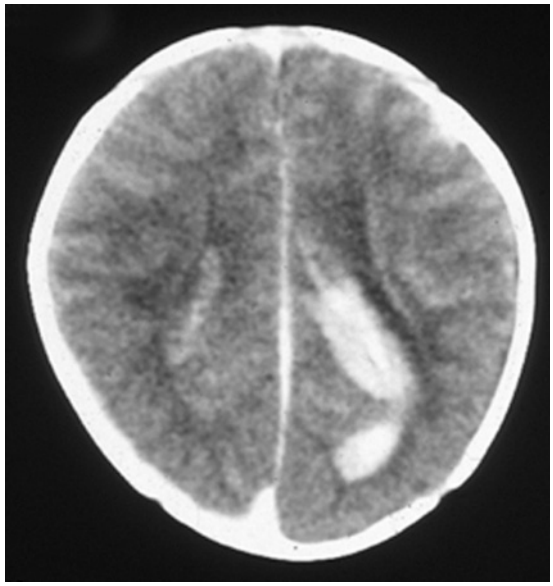

Figure 17.1 Intraventricular hemorrhage in a neonate with hemophilia.

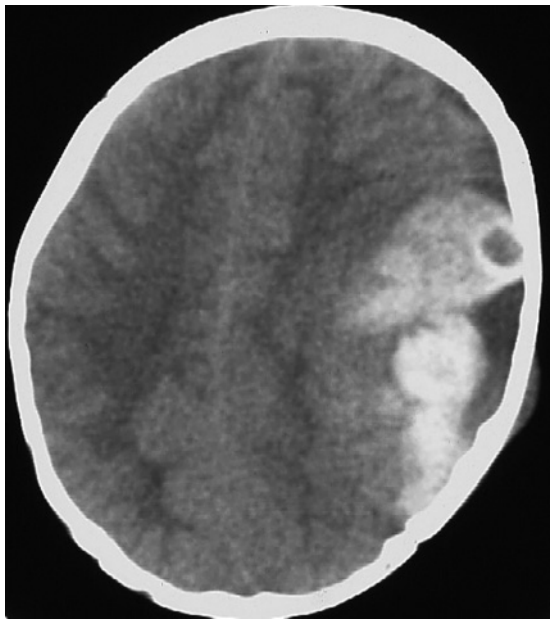

Figure 17.2 Subdural hemorrhage in a neonate with hemophilia.

Twenty of the 580 (3.4%) had an ICH compared with 0.11% of those without hemophilia. The rate was lower (1.9%) in those term babies with hemophilia without other complications or vacuum extraction [12].

The majority of bleeds occur within the first week of birth [6] and include excessive oozing from puncture sites, including heel-pricks, ECH such as subgaleal bleeds and cephalhematomas, and intracranial bleeding. Bleeding from the umbilical stump is rare but unusual bruising can be seen with minimal trauma (Figure 17.3) and large hematomas secondary to intramuscular vitamin K can occur.

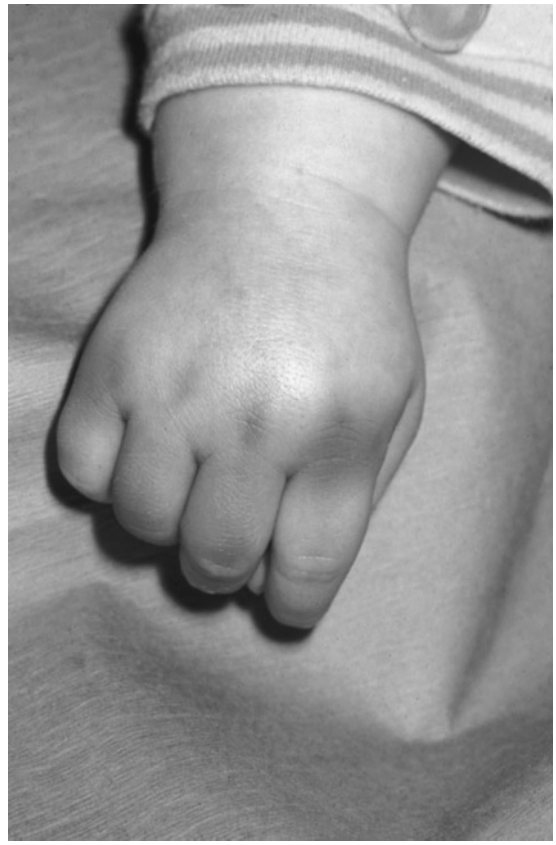

Figure 17.3 Bruising secondary to hand being held firmly for venipuncture in a neonate with severe hemophilia. (See also Plate 17.3.)

A recent review of neonatal bleeding in babies with hemophilia notes that the commonest site of bleeding is the cranium, with ICH accounting for 27% of all bleeds and ECH accounting for 13% [13,14]. However, in one Swedish series, 75% of all cranial hemorrhages were extracranial and it is possible that ECH is underreported [4]. Iatrogenic bleeding included bleeding from puncture sites (vascular, capillary, and intramuscular) in 16% of infants and from circumcision in 30% [13]. This latter figure will be considerably lower in countries where early circumcision is not customary. Umbilical stump bleeding was rare (6%) as were gastrointestinal/mouth bleeds, parenchymal bleeds, joint bleeds, or ecchymoses (<5%).

The incidence of ICH in neonates with hemophilia is reported as being between 1% and 4% [12,13] and is usually related to birth trauma regardless of the mode of delivery. The consequences can be devastating with death or subsequent neurologic deficit as the outcome [5,6,15,16]. ECH also occurs either alone or in conjunction with an ICH and can be life

threatening because of hypovolemic shock. A 22.8% mortality rate with subgaleal hemorrhages in all newborns has been reported [17]. Although the majority of bleeds occur and are identified before 1 week of age, many infants are now discharged from hospital early, some even within 24 h. Signs and symptoms of ICH can be nonspecific and include symptoms such as lethargy, poor feeding, and irritability. In those where the diagnosis of hemophilia is unsuspected, only general advice will have been given and the diagnosis of ICH may be delayed.

Raising the awareness of the need for full investigation of neonates with unexpected or unusual bleeding including liaison with a hematologist experienced in this area is the key to early identification of unsuspected hemophilia. Life-threatening bleeds can be treated appropriately and early diagnosis will help to prevent further bleeding or delay in treatment in the future. Neonatologists rarely come across babies with hemophilia and therefore are unfamiliar with their presentation in this period. Bleeding in the neonatal period, even in hemophilia, is rare and therefore most undiagnosed babies with hemophilia do not present at this time. Many hematologists who predominantly care for adults are also unaware of problems in the neonate or have not been involved in their care.

Investigation and management of a neonate with a positive family history of hemophilia

Perinatal management

The safe outcome of the neonate at risk of hemophilia is highly dependent on appropriate management during the perinatal period. Good communication between the involved obstetrician, hematologist, and pediatrician is crucial, particularly if the delivery is to take place at a site distant from a hemophilia center. If the genetic defect causing the hemophilia is known prior to the pregnancy, this broadens reproductive choices. Although the uptake of prenatal diagnosis in hemophilia is low, fetal sexing by ultrasound scanning will usually have been undertaken during the second trimester of pregnancy to determine if the fetus is male. However, this method can only reliably identify the sex of the fetus from no earlier than 13 weeks' gestation [18,19]. Fetal sex determination from maternal blood has been successful from the seventh week of gestation by testing circulating cell-free fetal DNA for Y-chromosome-specific sequences. This allows the choice of chorionic villous sampling where the fetus is male from 10 weeks' gestation [20]. Preimplantation genetic diagnosis is akin to very early prenatal diagnosis, eliminating the need for prenatal diagnosis and possible termination of the pregnancy. Molecular and cytogenetic techniques allow identification of abnormalities in embryos and only unaffected ones are implanted into the uterus [21].

Delivery

The optimal mode of delivery for an at-risk male fetus has been the subject of significant controversy but current practice in most centers is to consider vaginal delivery as the initial approach, unless there are specific obstetric contraindications. In order to avoid additional head trauma it is recommended that instrumental interventions including forceps, vacuum extraction, and the use of scalp electrodes are avoided and early recourse to cesarean delivery is advised where labor fails to progress. Although vacuum extraction should be avoided, where the head is deeply engaged in the pelvis, "lift-out" forceps may be less traumatic than cesarean section, provided the operator is experienced in the procedure [22]. These recommendations are based on findings from a small number of individual studies where cranial bleeding in the early neonatal period appeared to be strongly associated with trauma at the time of delivery, particularly with the use of vacuum extraction [6,9]. Nevertheless, it should be noted that in a review of 102 published cases of neonatal cranial bleeding in those with hemophilia, 19/47 (40%) followed apparently spontaneous vaginal delivery and such bleeds have also been recorded following cesarean section for obstetric reasons [13,23].

Diagnostic investigations

Following delivery, cord blood should be obtained for coagulation screening and factor VIII or IX assays. Testing cord blood avoids potential trauma to the neonate but care is required to avoid contamination with maternal blood or activation of the sample prior to testing. In the event that cord blood is not obtained, a venous sample should be obtained from the neonate as soon as possible after birth.

The hemostatic system in the neonate differs significantly from that observed in older children and adults and is often described as being physiologically immature at birth. This affects the levels of many procoagulant proteins and results in prolongation of baseline coagulation parameters including the activated partial thromboplastin time (aPTT) [24]. It is therefore important that all coagulation investigations are interpreted using reference ranges that take into account both the gestational and postnatal age of the infant. Such reference ranges are also machine and reagent specific and while larger laboratories may derive their own local reference ranges, smaller centers may be dependent on previously published ranges, which may not reflect current technology and must therefore be interpreted with care.

Hemophilia A and B classically result in an isolated prolongation of the aPTT and the diagnosis is confirmed by measurement of FVIII and FIX levels, respectively. In the neonate, factor VIII levels are within the normal adult range in both term and preterm infants whereas FIX levels are reduced to around 50% of adult values at term and are further reduced in preterm neonates [24,25]. It is therefore possible to confirm a diagnosis of hemophilia A in a neonate regardless

of the gestational age or the severity of the condition. It is also usually possible to diagnose severe and moderate hemophilia B, but confirmation of mild hemophilia B is complicated by overlap with normal values, necessitating repeat testing at around 6 months of age or molecular analysis if the genetic defect is known.

Vitamin K

Intramuscular vitamin K remains the preferred regimen for the prevention of vitamin K deficiency bleeding in many countries. The administration of vitamin K by this route should be delayed until diagnostic tests for hemophilia are completed. If hemophilia is confirmed or there is likely to be a delay in obtaining results, vitamin K should be administered according to an oral regimen. Heel-stab sampling for other neonatal screening procedures carries the risk of iatrogenic bleeding and should be performed with care.

Routine cranial scanning

One of the most devastating bleeds in the neonate with hemophilia is ICH. Early diagnosis will allow prompt treatment with the aim of stopping the bleed and therefore limiting the damage and long-term sequelae. Such bleeds can be diagnosed by cranial ultrasound, which is a noninvasive procedure that can be done at the cot-side. Although the majority of bleeds can be diagnosed by this method, subdural hematomas can be missed, as can posterior fossa hemorrhages. A normal scan therefore will not exclude all bleeds. It has been shown that in up to 10% of neonates without hemophilia, ICH can occur during birth [11,26]. In one study, all bleeds had resolved by 4 weeks and there were no sequelae [11]. In the other, only 0.4% developed symptoms in the first year of life [26]. It is not known, however, whether these children had an underlying coagulation or anatomic defect. Routine scanning may therefore pick up bleeds of uncertain significance and may miss some significant bleeds. It would seem appropriate to treat an ICH identified on routine scanning in a hemophilic neonate since the consequences can be serious or fatal, but whether all hemophilic neonates should have routine cranial ultrasound is not known. A survey of hemophilia centers in the UK regarding management of neonatal hemophilia showed that only 41% of those responding would routinely perform a cranial ultrasound on babies with severe hemophilia; 21% would only perform a cranial ultrasound in the presence of clinical signs suggestive of bleeding [27]. Despite the problems and uncertainties, it can be useful to perform a cranial ultrasound scan, particularly if there has been an instrumental delivery [10,28] remembering that if symptoms compatible with a bleed develop after a negative scan, a follow-up computed tomography (CT) or magnetic resonance imaging (MRI) scan should be performed.

Counseling

Once a diagnosis of hemophilia has been made it is important that the families of affected infants are referred promptly to their local hemophilia treatment center. Neonatal bleeding problems not infrequently present following discharge from hospital and it is therefore important that prior to discharge parents are adequately counseled regarding the diagnosis and are informed about potential problems, including those which could relate to major bleeding, particularly ICH.

Female carriers

Female infants who are potential carriers of hemophilia appear to be at low risk of bleeding during the neonatal period. Occasional cases will, however, have particularly low factor levels due to extreme lyonization or Turner's syndrome, and any abnormal bleeding should be appropriately investigated.

Investigation of abnormal bleeding in the absence of a positive family history

The hemorrhagic neonate

The majority of hemorrhagic problems observed during the neonatal period are a result of acquired hemostatic disorders. Thrombocytopenia and acquired coagulopathies because of disseminated intravascular coagulation, liver disease, and vitamin K deficiency are commonly observed in the context of underlying illness and in sick preterm neonates. This is in contrast to the typical presentation of an inherited coagulation disorder, which is more likely to be seen in a term infant with isolated bleeding problems.

Recognition of abnormal bleeding is crucial, particularly as signs are often subtle or nonspecific and it is vital that in the presence of unexplained bleeding appropriate investigations are initiated to exclude hemophilia and other inherited bleeding disorders. It is also important that coagulation investigations include specific factor assays and not just baseline coagulation screening tests, which can be misleading due to the physiologic prolongation of these parameters during the neonatal period. In the presence of major hemorrhage, disseminated intravascular coagulation (DIC) may coexist with an underlying inherited defect, which may further complicate the results obtained.

Problems with diagnosis

Unfortunately, the published literature highlights ongoing problems with delayed recognition and inadequate investigation of abnormal bleeding in this age group. In 1988, Yoffe reported that a diagnosis of hemophilia was delayed, in some cases for several months, in six of eight infants presenting with ICH [5]. More recently, in 2001, Myles reported two infants

presenting with ICH where neurosurgery was undertaken before a definitive diagnosis was reached [29].

Failure to initiate appropriate investigations has also been highlighted in a survey from the USA published in 1999 in which neonatologists and hematologists were questioned regarding how they would investigate neonates presenting with ICH. Among neonatologists only 23% reported that they would specifically request FVIII and FIX assays in a term neonate presenting with ICH and the figure dropped to 3% in preterm infants. The figures were higher when the same questions were asked of hematologists—64% and 39%, respectively, but in many centers hematologists may not be actively involved in initiating these investigations. In light of this, it is now advised by the Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation in the USA that all neonates with intracranial hemorrhage should be specifically investigated for the presence of an underlying bleeding disorder.

Treatment of hemophilia during the neonatal period

Choice of product

In the presence of acute bleeding or where prophylactic management is deemed necessary, treatment should be initiated with an appropriate factor concentrate. This applies regardless of severity, as desmopressin (DDAVP) is contraindicated during the neonatal period because of the risk of hyponatremia [30]. The choice of product should be governed by issues of safety and availability. In the developed world recombinant FVIII and FIX concentrates are now widely available and neonates should have the highest priority to receive these products [31]. This is based on the likelihood that recombinant technology will be associated with the lowest risk of transmitting viral infections or prions [31]. In those parts of the world where recombinant products are not available, high-purity, virucidally inactivated, plasma-derived products remain the preferred treatment. Where prothrombin complex concentrates (PCCs) are used for the treatment of hemophilia B it should be noted that an increased risk of DIC has been reported in neonates treated with these products.

As FVIII and FIX concentrates, particularly recombinant products, are not widely available outside hemophilia treatment centers it is important that arrangements are made to secure a supply of an appropriate product prior to the delivery of a potentially affected neonate. Although fresh-frozen plasma (FFP) is not recommended for the treatment of neonatal hemophilia, it may have a role in the presence of major hemorrhage where hemophilia is suspected but confirmatory investigations are not yet available or where other products are unavailable.

Dosing regimens

Dosing regimens for neonates are largely based on those used for older children and adults [32], as there is little published information available regarding the pharmacokinetics of replacement therapy in this age group. In view of this, careful monitoring of factor levels is likely to be particularly important. Some case reports in preterm infants with hemophilia A have shown that recovery of FVIII is similar to adults and older children but that the half-life is shorter, at 6–8 h [32–35]. In a single case report describing replacement therapy in a preterm neonate with hemophilia B, FIX recovery was similar to that seen in older children, whereas the half-life was lower at 6 h [33].

Prophylactic treatment

Given that the incidence of ICH in neonates with severe or moderate hemophilia is 1–4% and the outcome of such bleeds can be fatal or severely disabling, some have advocated prophylactic factor replacement for all known neonates with hemophilia and for those in whom the diagnosis is highly likely [36,37]. Prophylactic use of factor concentrate is well established in the older hemophiliac both for preventing spontaneous bleeds, particularly joint bleeds, and after injury before bleeding has taken place, especially when that injury could result in ICH. In the neonate, the head and brain have been subjected to significant stress but unlike traumatic head injuries the stress is physiologic. Nevertheless, ICH occurs and the risk is increased in deliveries requiring instrumentation or cesarean section during labor [6,9]. Although early studies suggested an association between early treatment and inhibitor development [38,39], this has not been confirmed in subsequent larger studies [40,41]. Current data indicate that the genetic mutation in the factor VIII gene is the most predictive parameter for inhibitor development. In those with a traumatic delivery, immediate treatment should be considered and in addition a high index of suspicion of ICH should be maintained. In a UK survey of hemophilia centers, only 19% of responders said they would consider prophylaxis in all severe cases but up to 62% following instrumental delivery [27]. In a survey in the USA, 89% of pediatric hematologists favored early prophylaxis with factor concentrates [42].

Hepatitis B vaccination

Even in cases where infants are receiving recombinant products, routine vaccination against hepatitis B continues to be recommended at the present time [31].

Conclusion

Hemophilia A and B are rare disorders but can present in the neonatal period with catastrophic bleeding. If the diagnosis is

missed, not only is appropriate treatment not instituted at the time, but further bleeds may occur with attendant morbidity or even mortality before the diagnosis is eventually made. Although there has been a significant improvement in making an early diagnosis, cases continue to be missed despite a positive family history or unusual bleeding in a well neonate. It is important to encourage liaison between obstetricians, neonatologists, and hematologists in the management of the known hemophilia carrier and between hematologists and neonatologists when abnormal bleeding in a neonate occurs. The importance of family history can be emphasized during genetic counseling of the mother so that appropriate information is given and help sought. Medical staff in attendance also need to be aware of the importance of a family history of bleeding. Written protocols for the management of the hemophilia carrier should be available in institutions where such mothers are delivered and protocols for the investigation of a bleeding neonate available in all neonatal units. Prospective studies collecting data on neonatal bleeding episodes, the contribution of routine cranial imaging, and analysis of early exposure to factor VIII concentrate and inhibitor development may help determine best management in the future.

References

1 Baehner RL, Strauss H. Hemophilia in the first year of life. *N Engl J Med* 1966; **275**: 524–8.

2 Chambost H, Gaboulaud V, Coatmelec B, Rafowicz A, Schneider P, Calvez T. What factors influence the age at diagnosis of hemophilia? Results of a French cohort study. *J Paediat* 2002; **141**: 548–52.

3 Conway JH, Hilgartner MW. Initial presentation of paediatric hemophiliacs. *Arch Pediatr Adolesc Med* 1994; **148**: 589–94.

4 Ljung R, Petrini P, Nilsson IM. Diagnostic symptoms of severe and moderate haemophilia A and B. *Acta Paediatr Scand* 1990; **79**: 196–200.

5 Yoffe G, Buchanan GR. Intracranial hemorrhage in newborn and young infants with hemophilia. *J Paediatr* 1988; **113**: 333–6.

6 Klinge J, Auberger K, Auerswald G, Brackmann HH, Mauz-Körholz Ch, Kreuz W. Prevalence and outcome of intracranial haemorrhage in haemophiliacs—A survey of the paediatric group of the German Society of Thrombosis and Haemostasis (GTH). *Eur J Pediatr* 1999; **158**: S162–5.

7 Pollmann H, Richter H, Ringkamp H, Jurgens H. *Eur J Pediatr* 1999; **158**: S166–70.

8 Giannelli F, Green PM. The molecular basis of haemophilia A and B. *Bailieres Haematology* 1996; **9**: 211–28.

9 Ljung R, Lindgren A-C, Petrini P, Tengborn L. Normal vaginal delivery is to be recommended for haemophilia carrier gravidae. *Acta Paediatr* 1994; **83**: 609–11.

10 Towner D, Castro MA, Eby-Wilkens E, Gilbert WM. Effect of mode of delivery in nulliparous women on neonatal intracranial injury. *N Engl J Med* 1999; **341**: 1709–14.

11 Whitby EH, Griffiths PD, Rutter S, *et al*. Frequency and natural history of subdural haemorrhages in babies and relation to obstetric factors. *Lancet* 2004; **363**: 846–51.

12 Tarantino MD, Gupta SL, Brusky RM. The incidence and outcome of intracranial haemorrhage in newborns with haemophilia: Analysis of the Nationwide Inpatient Sample database. *Haemophilia* 2007; **13**: 380–2.

13 Kulkarni R, Lusher JM. Intracranial and extracranial hemorrhages in newborns with hemophilia. *J Pediatr Hematol Oncol* 1999; **21**: 289–95.

14 Kulkarni R, Lusher J. Perinatal management of newborns with haemophilia. *Br J Haematol* 2001; **112**: 264–74.

15 Khair K, Baxter B, Fields P, Hann I, Leisner R. Intracranial haemorrhage in a tertiary pediatric centre: A survey of 100 children with severe haemophilia. *Haemophilia* 2002; **8**: 522–6.

16 Kletzel M, Miller CH, Becton D, Chadduck WM, Elser JM. Postdelivery head bleeding in hemophilic neonates. *Arch J Dis Child* 1989; **143**: 1107–10.

17 Plauche WC. Subgaleal haematoma. A complication of instrumental delivery. *J Am Med Assoc* 1980; **244**: 597–8.

18 Efrat Z, Akinfenwa OO, Nicolaides KH. First-trimester determination of fetal gender by ultrasound. *Ultrasound Obstet Gynecol* 1999; **13**: 305–7.

19 Whitlow BJ, Lazanakis MS, Economides DL. The sonographic identification of fetal gender from 11 to 14 weeks of gestation. *Ultrasound Obstet Gynecol* 1999; **13**: 301–4.

20 Bustamante-Aragones A, Rodriguez de Alba M, Gonzalez-Gonzalez C, *et al*. Foetal sex determination in maternal blood from seventh week of gestation and its role in diagnosing haemophilia in the foetuses of female carriers. *Haemophilia* 2008; **14**: 593–8.

21 Street AM, Ljung R, Lavery SA. Management of carriers and babies with haemophilia. *Haemophilia* 2008; **14**(Suppl. 3): 181–7.

22 Kadir RA, Economides DL. Obstetric management of carriers of haemophilia. *Haemophilia* 1997; **3**: 81–6.

23 Michaud JL, Rivard GE, Chessex P. Intracranial hemorrhage in a newborn with hemophilia following elective caesarean section. *Am J Pediatr Hematol Oncol* 1991; **13**: 473–5.

24 Andrew M, Paes B, Milner R, *et al*. Development of the coagulation system in the full-term infant. *Blood* 1987; **70**: 165–72.

25 Andrew M, Paes B, Milner R, *et al*. Development of the human coagulation system in the healthy premature infant. *Blood* 1988; **72**: 1998–2005.

26 Heibel M, Heber R, Bechinger D, Kornhuber HH. The early diagnosis of perinatal cerebral lesions in apparently normal full term newborns by ultrasound of the brain. *Neuroradiology* 1993; **35**: 85–91.

27 Chalmers EA. On behalf of the UKHCDO Paediatric Working Party. Management of neonates with inherited coagulation disorders. A survey of current practice. *Haemophilia* 2002; **8**: 488.

28 Smith AR, Leonard N, Kurth MH. Intracranial hemorrhage in newborns with hemophilia: The role of screening radiologic studies in the first 7 days of life. *J Pediatr Hematol Oncol* 2008; **30**: 81–4

29 Myles LM, Massicotte P, Drake J. Intracranial hemorrhage in neonates with unrecognised hemophilia A: A persisting problem. *Pediatr Neurosurgery* 2001; **34**: 94–7.

30 Williams MD, Chalmers, EA, Gibson BES. Guideline: The investigation and management of neonatal haemostasis and thrombosis. *Br J Haematol* 2002; **119**: 295–309.

31 Keeling D. Tait C. Makris M. Guideline on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders. A United Kingdom Haemophilia Center Doctors' Organisation (UKHCDO) guideline approved by the British Committee for Standards in Haematology. *Haemophilia* 2008; **14**: 671–84.

32 Rickard KA. Guidelines for therapy and optimal dosages of coagulation factors for treatment of bleeding and surgery in haemophilia. *Haemophilia* 1995; **1**(Suppl. 1): 8–13.

33 Kraft KE, Verlaak R, Van Heijst AFJ, *et al*. Management of haemophilia in three premature infants. *Haemophilia* 2008; **14**: 378–80.
34 Bidlingmaier C, Bergmann F, Kurnik K. Haemophilia A in two premature infants. *Eur J Pediatr* 2005; **164**: 70–2.
35 Gale RF, Hird MF, Colvin BT. Management of a premature infant with moderate haemophilia A using recombinant factor VIII. *Haemophilia* 1998; **4**: 850–3.
36 Berry E. Intracranial haemorrhage in the haemophiliac neonate—the case for prophylaxis. Available at: http://www.haemophilia-forum.org/lock/Discussion/990426.htm
37 Buchanan GR. Factor concentrate prophylaxis for neonates with haemophilia. *J Pediatr Hematol Oncol* 1999; **21**: 254–9.
38 Lorenzo JI, López A, Altisent C, Aznar JA. Incidence of factor VIII inhibitors in severe haemophilia: The importance of patient age. *Br J Haematol* 2001; **113**: 600–3.
39 Van der Bom JG, Mauser-Bunschoten EP, Fischer K, van den Berg HM. Age at first treatment and immune tolerance to factor VIII in severe haemophilia. *Thromb Haemost* 2003; **89**: 475–9.
40 Chalmers EA, Brown SA, Keeling D, *et al*. Paediatric Working Party of UKHCDO. Early factor VIII exposure and subsequent inhibitor development in children with severe haemophilia A. *Haemophilia* 2007; **13**: 149–55.
41 Gouw SC, van der Bom JG, Marijke van den Berg H. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: The CANAL cohort study. *Blood* 2007; **109**: 4648–54.
42 Kulkarni R, Lusher JM, Henry RC, Kallens DJ. Current practices regarding new-born intracranial haemorrhage and obstetrical care and mode of delivery of pregnant haemophilia carriers: A survey of obstetricians, neonatologists and haematologists in the United States, on behalf of the National Hemophilia Foundation's Medical and Scientific Advisory Council. *Haemophilia* 1999; **5**: 410–15.

18 Work-up of a bleeding child

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Introduction

The hemostatic system consists of a regulated balance of procoagulant (forces that promote hemostatic plug formation at sites of vessel injury), anticoagulant (forces that inhibit coagulation), and fibrinolytic forces (forces that dissolve hemostatic clots). Disturbance of this balance may result in abnormal bleeding or clotting. A hemostatic plug is composed primarily of platelets and fibrin. Excessive bleeding may reflect quantitative and/or qualitative abnormalities of platelets, coagulation proteins, and/or components of the vessel wall. These abnormalities may be either inherited (congenital) or acquired.

The work-up of a child referred because of unusual or excessive bleeding should always include (i) a comprehensive medical (and specific bleeding) history; (ii) a detailed family history including whether there is consanguinity; (iii) a detailed physical examination; and (iv) selected laboratory tests. Results of laboratory tests need to be interpreted in relation to the age of the child as many coagulation parameters are affected by a child's age, particularly during the first year of life [1–3]. The above work-up should allow an accurate diagnosis to be made in children referred for evaluation of abnormal bleeding.

This review begins with a brief description of platelet plug formation in normal hemostasis and the interpretation of common laboratory tests. Subsequently, a practical approach to the evaluation of a child referred because of abnormal bleeding, including a commentary on specific hemostatic laboratory tests, is presented. Some selected common, as well as rare, inherited bleeding disorders are also discussed.

Platelet plug formation

The initiating event in hemostatic plug formation is the adhesion of circulating platelets to exposed subendothelium. The high-molecular-weight hemostatic multimeric forms of von Willebrand factor (VWF) play a critical role in this first event by linking platelets through the glycoprotein GPIbα component of the GPIb-IX-V receptor to collagen in the subendothelium [4]. Adhesion is further augmented by GPIa-IIa (integrin $\alpha_2\beta_1$) and GPVI receptors on platelets which also bind to collagen in the exposed subendothelium. As a result of this initial adhesive process, platelets become activated, resulting in the release of multiple proteins and ADP, and formation of thromboxane A_2. This then results in recruitment of additional platelets to the site of vessel injury, platelet to platelet aggregation, and the formation of a hemostatic plug (primary hemostasis). Key mediators in platelet aggregation include platelet GPIIb-IIIa receptors (integrin $\alpha_{IIb}\beta_3$) with the ligands fibrinogen and VWF (Figure 18.1), the latter being of greater importance under conditions of high shear. Activated platelets provide the anionic phospholipid surface necessary for the tenase and prothrombinase complexes to accelerate coagulation (secondary hemostasis). The platelet plug is then stabilized by the formation of cross-linked fibrin strands, a process that involves activated factor XIII.

Evaluation of the child with abnormal bleeding or a suspected bleeding disorder

Medical history, including bleeding history

The initial evaluation of a child referred because of a suspected bleeding disorder should include a detailed medical and bleeding history. The medical history should emphasize the child's age, whether or not the child is ill, the type of bleeding symptoms that the child is experiencing, and all medications, including herbal supplements, that the child is taking.

The child's age is extremely important since bleeding disorders presenting in newborn infants are significantly distinct from those encountered in older infants and children [5].

It is useful to separate patients into those who are ill and those who are well; the former group often includes children in hospital who have acquired bleeding disorders secondary to a variety of conditions, e.g., sepsis, renal, and/or liver disease. In such patients, several components of the hemostatic system may be perturbed (platelets, procoagulant, and anticoagulant proteins, and components of the fibrinolytic system), which may together contribute to abnormal bleeding [6–8]. In contrast, abnormal bleeding in otherwise well infants and children is more likely to reflect underlying single defects (inherited or acquired) of platelets or coagulation proteins.

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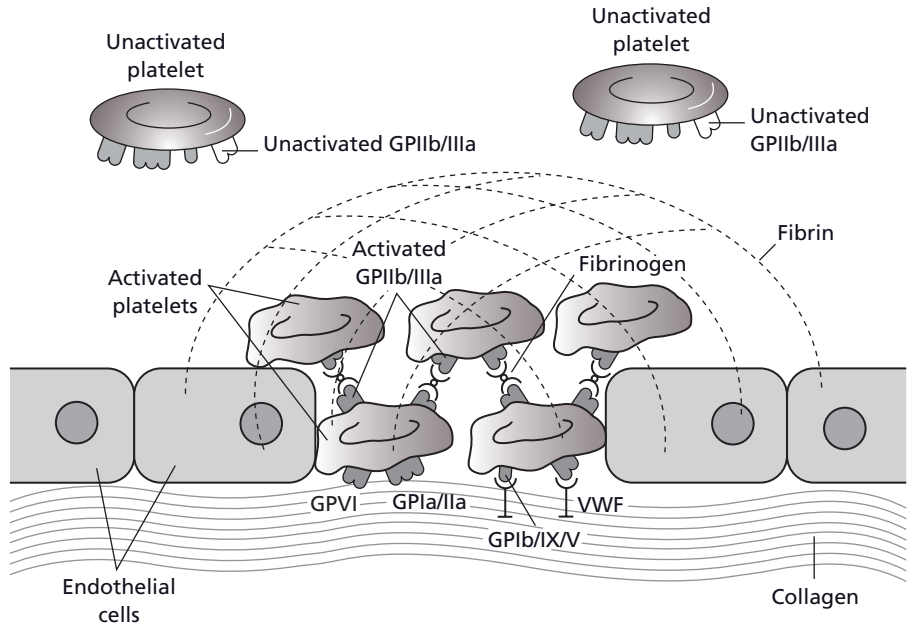

Figure 18.1 Schematic representation of a hemostatic plug. See text for details.

The type of bleeding is of great value in making a diagnosis. Mucocutaneous bleeding (easy bruising, recurrent and/or prolonged epistaxis, menorrhagia, and prolonged bleeding following cuts and/or dental extractions) is the hallmark of inherited or acquired disorders of primary hemostasis [platelet disorders and von Willebrand disease (VWD)]. In contrast, disorders of secondary hemostasis (coagulation factor deficiencies) usually present with more internal or deep bleeding (bleeds into joints and muscles as well as intracranial and surgical bleeding). Finally, disorders of the fibrinolytic system present with delayed surgical type bleeding, defective wound healing, and characteristically in newborn infants with bleeding from the umbilical stump.

The combination of the child's age along with the site and pattern of bleeding may yield important clues regarding an underlying etiology. For example, bleeding from multiple sites in an ill child should raise the suspicion of disseminated intravascular coagulation (DIC), whereas a history of bleeding into a joint in a boy would be very suggestive of hemophilia A [factor VIII (FVIII) deficiency] or B (FIX deficiency). Petechiae in an otherwise well child after a viral infection may indicate idiopathic thrombocytopenic purpura (ITP). A history of prolonged bleeding from the umbilical cord with delayed wound healing in a newborn infant is suggestive of FXIII deficiency, afibrinogenemia, or α_2-antiplasmin deficiency [9]. Excessive menstrual bleeding in an adolescent girl may be the first sign of VWD. Intracranial hemorrhage, if occurring in the newborn period, may be the first clue to an underlying severe coagulation bleeding disorder such as FXIII, FX, FIX, FVIII, FVII, FV, or fibrinogen deficiency. Other causes of intracranial hemorrhage in a newborn infant include birth trauma, DIC, congenital thrombocytopenia, and neonatal alloimmune thrombocytopenia [10].

Despite the importance of the bleeding history, it is often poorly taken, leading in some cases to excessive and unnecessary investigations. The use of defined pediatric bleeding questionnaires/scores is useful as they are more likely to differentiate a child who is a "bleeder" and deserves to be investigated from the "nonbleeder" who may not need any investigations. Standardized, quantitative, and semiquantitative questionnaires, both general [11–14] and organ specific [e.g., an epistaxis score [15] or a pictorial blood assessment chart (PBAC) for evaluation of menorrhagia] [16], facilitate a systematic, comprehensive, and discriminatory collection of information as opposed to a potentially superficial and subjective bleeding history often taken by many physicians. A number of disease- or organ-specific pediatric assessment tools have been developed [11,15,17].

It should be recognized that young children, because of their age, may not have had sufficient hemostatic challenges to experience symptoms that would classify them as "bleeders;" repeat assessments over time may therefore be necessary, particularly if there is a history of unusual or prolonged bleeding, or if the family history is positive for abnormal bleeding.

Family history

The family history may provide important clues regarding potential inheritance of an underlying bleeding disorder, e.g., a sex-linked inheritance pattern would be suggestive of hemophilia or Wiskott–Aldrich syndrome; an autosomal dominant

inheritance pattern would be in keeping with type 1 VWD and some platelet function disorders. Consanguinity in a family is suggestive of autosomal recessive disorders (e.g., type 2N and type 3 VWD, most platelet function disorders together with all factor deficiencies with the exception of hemophilia).

One example in which the family history can be very informative is the rare type 2N VWD variant [18]. In this condition, circulating FVIII coagulant levels (FVIII:C) are disproportionately low in comparison with circulating VWF antigen (VWF:Ag) and ristocetin cofactor (VWF:RCo) levels, because of an abnormality in VWF that results in a markedly decreased affinity for FVIII therewith causing enhanced FVIII:C clearance [19]. Patients with type 2N VWD may be labeled incorrectly as having mild/moderate hemophilia A [20] with potentially significant adverse effects if bleeding episodes are treated with a high-purity FVIII concentrate (devoid of functional VWF) rather than with a VWF-containing concentrate. The differing inheritance patterns for type 2N VWD (autosomal recessive) versus hemophilia (X-linked) is a useful diagnostic clue.

In general, there is little racial or ethnic predisposition to bleeding disorders with the major exception that autosomal recessive bleeding disorders are more common in populations where consanguinity is common. In addition, there are a few bleeding disorders which are more common in certain populations: FXI deficiency among Jews of Ashkenazi (European) origin and as well among the Basque population of southwestern France and north-eastern Spain [21]. Although not a bleeding disorder, FXII deficiency is thought to be more common in persons from China and eastern Asian countries [22,23]. The family and the ethnic history of a patient should therefore be taken into consideration when deciding about specific laboratory testing.

Physical examination

The physical examination is often of limited use in the diagnosis of children with suspected bleeding disorders. If present, the finding of purpura and/or petechiae in areas other than the lower limbs should raise the suspicion of an acquired (e.g., ITP) or congenital platelet disorder. In a boy, evidence of current or past bleeding into joints is suggestive of a diagnosis of hemophilia. Certain physical findings may point to specific, albeit relatively rare, bleeding disorders, e.g., eczema in patients with Wiskott–Aldrich syndrome, telangiectasias in patients with hereditary hemorrhagic telangiectasia, oculocutaneous albinism in patients with Hermansky–Pudlak or Chediak–Higashi syndromes, skeletal abnormalities in patients with thrombocytopenia absent radius (TAR) syndrome, joint laxity and scarring in patients with Ehlers–Danlos syndrome, and cataracts and/or hearing loss in some patients with inherited MYH9-related macrothrombocytopenias [24].

Laboratory evaluation

Laboratory evaluation should begin with screening laboratory tests unless the history and available laboratory results already performed are strongly suggestive of a specific bleeding disorder.

Screening coagulation tests should include a complete blood count (including a platelet count), examination of a blood smear, an activated partial thromboplastin time (aPTT), and the prothrombin time (PT) reflected by the international normalized ratio (INR). Additional tests that can be considered as screening studies include a fibrinogen level, and a thrombin time (TT). In the event of a prolonged TT a reptilase time is useful as reptilase, similar to thrombin, clots fibrinogen but, unlike thrombin, is not affected by heparin. Consequently, the reptilase time is useful to distinguish heparin contamination from afibrinogenemia (absence of fibrinogen) and dysfibrinogenemia (abnormally functioning fibrinogen).

If the child has a history of mucocutaneous bleeding, a screen to confirm or refute a diagnosis of VWD should be initiated. This should include measurements of FVIII:C, VWF:Ag, and VWF:RCo, and, if available, measurement of the closure time using the Platelet Function Analyzer, PFA-100® (see below). VWF multimer analysis is usually not done as part of initial screening tests as it can be done at a later date should initial studies suggest VWD. The skin bleeding time (see below) is no longer recommended as part of an initial hemostatic screen.

The result of screening laboratory tests taken together with the child's medical and family history should be used to decide about repeat testing or further specialized tests (e.g. platelet aggregation tests, platelet flow cytometry, platelet electron microscopy, and immunofluorescence studies for nonmuscle myosin heavy-chain IIA clumps). Three laboratory tests/scenarios deserve special commentary because of their importance in the context of evaluation of the child with abnormal bleeding; these are described below.

Enumeration of the circulating platelet count

The platelet count is typically measured in a sample of venous blood collected into ethylenediaminetetraacetic acid (EDTA) anticoagulant. Automated blood cell counters are usually more accurate and precise than manual platelet counting; the former are thought to have coefficient of variations (CVs) of <3% while the latter have a typical interobserver CV in the range of 10–25% [25]. However, at low platelet counts ($<20 \times 10^9$ platelets/L), a manual platelet count is more accurate and is recommended [26].

Examination of the blood smear is a vital component of the assessment of a bleeding disorder and cannot be emphasized enough. It allows for the determination of pseudothrombocytopenia in samples collected into EDTA anticoagulant owing to EDTA-induced *in vitro* platelet agglutination

[27]. Pseudothrombocytopenia can be prevented by using a different anticoagulant (e.g., citrate or heparin) or by preparing a blood film directly from a heel puncture. Examination of the blood smear also allows giant platelets (platelets larger than approximately 13 fL) to be visualized [24,28,29]. This is important as automated counters may exclude giant platelets, resulting in a spurious finding of thrombocytopenia [30]. Additionally examination of a blood film allows a few rare diagnoses to be made: Gray platelet syndrome, a condition in which platelets appear pale gray, and MYH9-related macrothrombocytopenias (formerly referred to as May Hegglin anomaly, Sebastian, Fechtner, or Epstein syndromes) in which leukocyte inclusion bodies (Döhle-like bodies) can sometimes be seen [31].

Examination of the blood film may also reveal red blood cell schistocytes (fragments), a clue to the diagnosis of hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and DIC; the latter being additionally characterized by the presence of a prolonged aPTT and INR.

Bleeding time and PFA-100®

The skin bleeding time is one of the most established screening tests of global platelet function used to investigate bleeding disorders, and template devices are available for use in children and newborn infants as well as in adults [3,32]. The bleeding time is not prolonged in patients with hemophilia, may be prolonged in patients with type 1 VWD, and is prolonged in some patients with platelet function disorders. It has become recognized, however, that the use of the bleeding time as a routine screening test is not warranted, particularly in young children. The test is highly operator dependent, invasive, poorly reproducible, and a poor predictor of bleeding risk [33].

The platelet function analyzer (PFA-100®) is a more recently developed *in vitro* (noninvasive) measure of primary, platelet-related hemostasis. This device has been demonstrated to be a more useful screening test for VWD than the bleeding time [11,25]. The test is quantitative, simple, rapid, and reproducible, and measures, under high-shear conditions, the time (closure time) taken for a platelet plug to occlude, or close, a microscopic aperture in a membrane coated with platelet agonists [34]. Like the bleeding time, the closure time is not prolonged in persons with hemophilia [35]. Unfortunately, most investigators have found that the PFA-100®, like the bleeding time, lacks specificity and sensitivity for detecting mild platelet function disorders [36–38]. It, like the bleeding time, is, however, sensitive to severe platelet function disorders such as Glanzmann thrombasthenia. When interpreting the results of the PFA-100® it should be recognized that the PFA-100® is affected by many variables (platelet count, hematocrit, and medications).

The aPTT: prolongation does not necessarily mean a bleeding disorder

A prolonged aPTT is a very commonly encountered laboratory abnormality. A prolonged aPTT may truly indicate an underlying bleeding disorder (e.g., hemophilia, VWD, or other factor deficiencies). Alternatively, there are multiple etiologies for a prolonged aPTT that are not associated with an increased risk of bleeding. These include the presence of antiphospholipid antibodies, FXII deficiency, and technical problems with blood collection and processing. The latter includes heparin contamination of blood samples.

Many viral and bacterial infections in childhood may induce the production of antiphospholipid antibodies. These tend to be benign and transient; lasting the usual duration of antibodies (2–3 months). These antibodies are likely to be the most common etiology in children referred for evaluation for an elevated aPTT [39]. The antibodies, other than prolonging the aPTT, are generally not clinically significant and unlikely to cause bleeding. A rare exception to this is when the antibody is additionally directed against prothrombin (FII) and results in consumption of FII [40]. Consequently, in a child with a combination of bruising and antiphospholipid antibodies a FII level should be obtained.

Homozygous or heterozygous deficiency of FXII (Hageman factor) similarly can prolong the aPTT. In the case of homozygous/compound heterozygous FXII deficiency the aPTT can be greatly prolonged (>100 s). Yet, despite this, the condition is not associated with bleeding manifestations. In fact there is some evidence that a deficiency of FXII can be thrombogenic [41].

Technical problems with blood collection and processing may prolong the aPTT without affecting a child's risk of bleeding. These preanalytic variables can include collecting too much or too little blood leading to an inappropriate ratio of blood to the anticoagulant reagent (in most cases citrate) [39]. Delays in sample processing and inappropriate storage of blood may also lead to a falsely elevated aPTT. Lastly, contamination of blood samples by heparin is a commonly encountered problem, especially in ill infants and children in intensive care units. In a hospital setting it is usually the most frequent cause of a prolonged aPTT. Heparin binds to antithrombin present in plasma samples, thereby enhancing inactivation of thrombin and FXa and, to a lesser extent, FIXa, FXIa, and FXIIa. At very high plasma heparin concentrations, the PT is also prolonged. A prolonged aPTT as a result of heparin contamination will also result in a prolonged TT. Heparin contamination in a plasma sample can be confirmed by normalization of the TT in the presence of protamine sulfate, which acts to neutralize any heparin present in the sample [42]. Alternatively, the snake venom reptilase (from *Bothrops atrox*), which is not inhibited by heparin, can be used instead of thrombin when measuring the TT. An abnormal TT but normal reptilase (clotting) time indicates heparin contamination [43]. If necessary, heparin can be removed

or neutralized in several ways [44]; this allows individual clotting factor levels to be measured in heparin-containing specimens.

Common bleeding disorders

In our large pediatric bleeding disorders clinic, the three commonest inherited bleeding disorders are hemophilia A or B, VWD, and inherited platelet function defects. These three are discussed below. Less common are rare congenital coagulation factor deficiencies, e.g., FXI, FVII, FXIII, or fibrinogen.

Hemophilia

The diagnosis of severe hemophilia is usually made within the first year of life because of testing at or around the time of birth in boys with a positive family history of the disorder, or because of abnormal bruising/bleeding [e.g., with blood sampling, with intramuscular injections (vitamin K, vaccines) or post circumcision]. The aPTT is always prolonged in boys with severe and moderate hemophilia, but can be normal in boys with mild hemophilia. It is therefore strongly recommended that, if a diagnosis of hemophilia is suspected, FVIII (or FIX levels) be specifically measured together with VWF levels as type 3 VWD may present with similar clinical findings (as hemophilia) and with a low FVIII level.

The diagnosis of mild/moderate hemophilia is often delayed and may occur only after investigation for unusual bleeding following invasive procedures (e.g., tonsillectomy). It is important to note that levels of FIX, like other vitamin K-dependent factors, are reduced in early life and only reach adult levels by about 6 months of age [2,32] (Figure 18.2). Consequently, a young child may be falsely labeled as having moderate or mild hemophilia B whereas 6–12 months later his FIX levels might place him in the category of mild hemophilia or potentially even normal. Repeat testing after an interval of 6–12 months may therefore be appropriate in cases of a low FIX level. In contrast, patients with mild hemophilia A can reliably be diagnosed at any time since plasma FVIII levels do not vary with age.

von Willebrand disease

von Willebrand disease is caused by quantitative (types 1 and 3) and/or qualitative defects (type 2) of the multimeric glycoprotein VWF [45]. A current classification of VWD is presented in Table 18.1. The most common and mildest type of VWD (type 1) is the most difficult to diagnose and to distinguish from normal. Repeat testing is often required to determine whether an individual has type 1 VWD or is normal. Part of the problem is that the 95% confidence interval for VWF in the normal population can vary between 50% and 200% of the mean level, and there is significant temporal variability in VWF levels [46]. In addition, the mean VWF:Ag and VWF:RCo levels are 20–25% lower in blood group O than in nonblood group O subjects [11,47].

In contrast, it is much easier to make a diagnosis of VWD in patients with more severe forms of VWD, e.g., type 2 and type 3 VWD. Type 3 VWD is particularly easy to diagnose because of the combination of its severity, the common finding of parental consanguinity, and absent levels of VWF. Distinguishing the various subtypes of type 2 VWD (Table 18.1) is, however, problematic. It can usually be achieved by laboratories capable of accurately measuring the following: FVIII:C, VWF:Ag, VWF:RCo, VWF multimer analysis, and ristocetin-induced platelet agglutination (RIPA) testing. In some cases, genetic testing may be required to confirm the subtype of type 2 VWD.

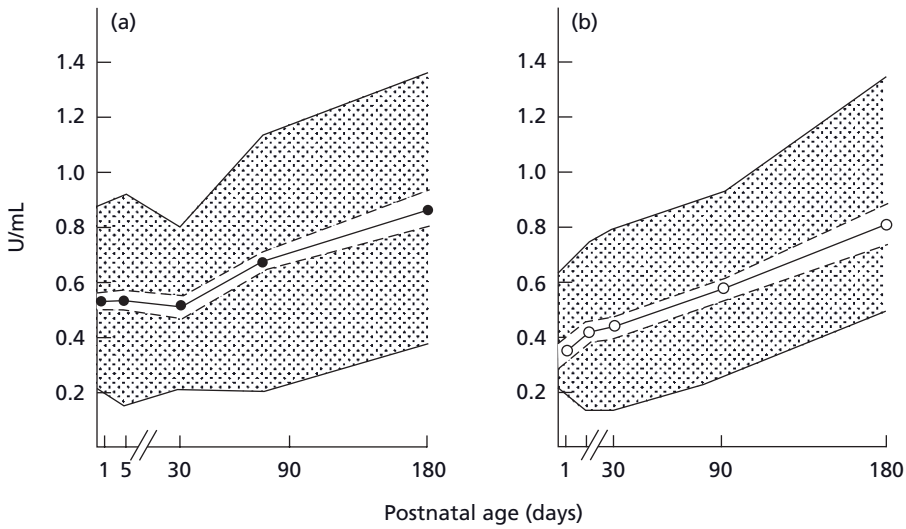

Figure 18.2 Factor IX levels in healthy full-term infants (a) and healthy premature infants (b) during the first 6 months of life. (a) Factor IX levels in 118 full-term infants. (b) Factor IX levels in 137 healthy premature infants (30–36 weeks' gestation). The inner line represents the mean values, the inner clear area the 95% confidence interval, and the shaded area 95% of all values. The mean adult factor IX levels are 1.09 ± 0.27 U/mL. Figure adapted from refs 1 and 2.

Table 18.1 Classification of von Willebrand disease.

Type	Frequency (%)	Comment
1	~80	Partial quantitative deficiency of VWF
2	15–20	Qualitative defects of VWF
2A		Variants with decreased VWF-dependent platelet adhesion associated with a deficiency of high-molecular-weight VWF multimers
2B		Variants with an increased affinity for platelet glycoprotein Ib leading to increased platelet binding, thrombocytopenia, and a reduction of high-molecular-weight VWF multimers
2M		Variants with decreased VWF-dependent platelet adhesion associated without a deficiency of high-molecular-weight VWF multimers
2N		Variants with markedly decreased binding affinity for FVIII
3	Rare	Virtually complete absence of VWF

FVIII, factor VIII; VWF, von Willebrand factor.
Reproduced from [45] with permission.

A rare condition that resembles type 2B VWD is platelet-type or pseudo-VWD caused by mutations in the platelet GPIbα gene causing enhanced binding of GPIbα to VWF [48,49]. Enhanced RIPA is observed in both type 2B VWD and platelet-type VWD. Platelet agglutination studies at low concentrations of ristocetin (0.5 U/mL) using normal platelets and patient's plasma, or patient's platelets and normal plasma, allow one to differentiate between the two conditions [50].

Platelet disorders

Platelet disorders encompass a wide spectrum of primary hemostatic bleeding disorders, which may be inherited or acquired and include quantitative (e.g., thrombocytopenia absent radius), qualitative (e.g., Glanzmann thrombasthenia), or mixed quantitative/qualitative defects (e.g. Bernard–Soulier syndrome, which involves both thrombocytopenia and platelet dysfunction) [51,52]. Like VWD, platelet disorders present primarily with mucocutaneous bleeding manifestations.

Acquired causes of thrombocytopenia and/or platelet dysfunction in children (e.g., DIC, ITP, HUS, TTP, uremia, and liver disease) are common [53] but will not be discussed here.

In the view of some experts, inherited disorders of platelet function may be more common in the general population than has previously been appreciated [54,55]. Such patients may remain undiagnosed and are simply considered to be "easy bruisers/bleeders." These inherited platelet disorders can be distinguished by the presence/absence of thrombocytopenia, the size and appearance of platelets, the pattern of inheritance, the presence/absence of other physical findings, and ultimately on the mechanism of the platelet dysfunction (Table 18.2).

Screening laboratory tests for inherited platelet disorders should include a complete blood count with accurate enumeration of the platelet count and a careful examination of the peripheral blood film noting whether leukocyte (Döhle-like) inclusion bodies are present. The presence or absence of thrombocytopenia and the size and appearance of platelets are very useful diagnostic clues to the underlying platelet disorder (refer to Table 18.2 for a list of platelet disorders associated with large, small, or normal-sized platelets). In patients in whom the medical and/or family history is suggestive of a platelet disorder, and in whom an alternative explanation for bleeding symptoms such as VWD has been excluded, platelet aggregation studies should be performed. The panel of agonists commonly used to assess aggregration includes ADP, collagen, arachidonic acid, a thromboxane A_2 analogue (e.g., U46619), epinephrine, and ristocetin [55]. In Glanzmann thrombasthenia, platelets respond only to ristocetin, whereas in Bernard–Soulier syndrome platelets respond to all agonists except ristocetin. Flow cytometry for the quantitation of GPIIb-IIIa and GPIb–IX-V receptors on platelets is useful to confirm these two respective diagnoses.

Dense (δ) granule platelet storage pool disorders can be identified using whole-mount electron microscopy or chemiluminescence, whereas transmission electron microscopy is useful to exclude α-granule disorders such as gray platelet syndrome [56,57]. Immunofluorescence testing of neutrophils to demonstrate abnormal clumping of nonmuscle myosin heavy-chain IIA seen in MYH9-related disorders is becoming a useful, although not widely available, laboratory test [58].

Finally, genetic testing can be performed for some inherited platelet disorders where gene defects have been identified (e.g., Glanzmann thrombasthenia, Bernard–Soulier syndrome, Wiskott–Aldrich syndrome, Hermansky–Pudlak syndrome, Paris–Trousseau syndrome, MYH9-related macrothrombocytopenias, and others) [59]. Such DNA analyses of inherited platelet defects are limited to specialized laboratories.

Conclusion

The correct and timely diagnosis of an inherited bleeding disorder in a child is important. This goal can best be achieved by combining a careful history, a detailed physical examination, and selected laboratory tests. Once a diagnosis is made, the child (if of an appropriate age) and/or parents or guardians can be counseled about the severity and anticipated natural history of the disorder plus available management options, both specific (e.g., factor concentrate therapy in boys with hemophilia) and nonspecific (e.g., DDAVP and/or antifibrinolytic therapy in children with type 1 VWD or congenital platelet disorders).

Table 18.2 Classification of platelet disorders.

Diagnosis	Key clinical/laboratory findings	Inheritance	Thrombocytopenia	Platelet size
Disorders of platelet adhesion/aggregation				
Bernard–Soulier syndrome	GPIb-IX-V deficiency	AR	Mild to moderate	Large
Glanzmann thrombasthenia	GPIIb-IIIa deficiency	AR	None	Normal
Disorders of platelet signaling				
Specific platelet receptor abnormalities	Collagen (GPVI/GPIa-IIa)	AD	None	Normal
	ADP ($P2Y_{12}$)	AD	None	Normal
	Thromboxane A_2	AD	None	Normal
Disorders of platelet granule secretion				
Defects of dense (δ) granules (isolated or as part of the following syndromes)				
Hermansky–Pudlak syndrome	Oculocutaneous albinism, granulomatous colitis, pulmonary fibrosis	AR	None	Normal
Chediak–Higashi syndrome	Oculocutaneous albinism, immunodeficiency, neurological dysfunction, lymphoproliferation	AR	None	Normal
Defects of α granules (isolated or as part of the following syndromes)				
Gray platelet syndrome	Defective retention of α-granule proteins, myelofibrosis	AR	Mild to moderate	Large
Quebec platelet disorder	Increased platelet u-PA, enhanced α granule protein degradation	AD	None to mild	Normal
ARC syndrome	Arthrogryposis, renal disease, cholestasis	AR	None	Large
Disorders of platelet production				
Thrombocytopenia with absent radius syndrome (TAR)	Shortened/absent radii	AR	Severe	Small to normal
Congenital amegakaryocytic thrombocytopenia	TPO-receptor mutation, absence of megakaryocytes, pancytopenia	AR	Severe	Normal
Wiskott–Aldrich syndrome	Immunodeficiency, eczema, lymphoma	X-linked	Severe	Small
X-linked thrombocytopenia	No immune problems	X-linked	Severe	Small
MYH9-related disorders[a]	Leukocyte inclusion (Döhle-like) bodies, nephritis; hearing loss; cataracts	AD	Mild-Moderate	Large
Paris–Trousseau/Jacobsen syndrome	Cardiac and facial defects, mental retardation, giant α granules	AD	Mild-Moderate	Large
Other				
Scott syndrome	Decreased platelet procoagulant activity	AR	None	Normal

AD, autosomal dominant; AR, autosomal recessive; u-PA, urokinase-type plasminogen activator.
[a]Formerly referred to as May–Hegglin anomaly, Sebastian, Fechtner, Epstein syndromes.

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References

1 Andrew M, Paes B, Milner R, *et al*. Development of the human coagulation system in the full-term infant. *Blood* 1987; **70**: 165–72.
2 Andrew M, Paes B, Milner R, *et al*. Development of the human coagulation system in the healthy premature infant. *Blood* 1988; **72**: 1651–7.
3 Andrew M, Vegh P, Johnston M, *et al*. Maturation of the hemostatic system during childhood. *Blood* 1992; **80**: 1998–2005.
4 Furie B, Furie BC. Mechanisms of thrombus formation. *N Engl J Med* 2008; **359**: 938–49.
5 Zipursky A, deSa D, Hsu E, *et al*. Clinical and laboratory diagnosis of hemostatic disorders in newborn infants. *Am J Pediatr Hematol Oncol* 1979; **1**: 217–26.
6 Levi M, Ten Cate H. Disseminated intravascular coagulation. *N Engl J Med* 1999; **341**: 586–92.
7 Lisman T, Leebeek FW, de Groot PG. Haemostatic abnormalities in patients with liver disease. *J Hepatol* 2002; **37**: 280–7.
8 Monagle P, Andrew M. Acquired disorders of hemostasis. In: Nathan DG, Orkin SH, Ginsburg D, Look AT (eds). *Nathan and Oski's Hematology of Infancy and Childhood*, Vol. 2, 6th edn. Philadelphia: WB Saunders; 2003: 1631–67.
9 Acharya SS, DiMichele DM. Rare inherited disorders of fibrinogen. *Haemophilia* 2008; **14**: 1151–8.
10 Dreyfus M, Kaplan C, Verdy E, *et al*. Frequency of immune thrombocytopenia in newborns: A prospective study. Immune Thrombocytopenia Working Group. *Blood* 1997; **89**: 4402–6.

11 Dean JA, Blanchette VS, Carcao MD, *et al*. von Willebrand disease in a pediatric-based population—Comparison of type 1 diagnostic criteria and use of the PFA-100 and a von Willebrand factor/collagen-binding assay. *Thromb Haemost* 2000; **84**: 401–9.

12 Rodeghiero F, Castaman G, Tosetto A, *et al*. The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: An international, multicenter study. *J Thromb Haemost* 2005; **3**: 2619–26.

13 Tosetto A, Castaman G, Rodeghiero F. Bleeding scores in inherited bleeding disorders: Clinical or research tools? *Haemophilia* 2008; **14**: 415–22.

14 Rodeghiero F, Kadir RA, Tosetto A, James PD. Relevance of quantitative assessment of bleeding in haemorrhagic disorders. *Haemophilia* 2008; **14**(Suppl. 3): 68–75.

15 Katsanis E, Luke KH, Hsu E, Li M, Lillicrap D. Prevalence and significance of mild bleeding disorders in children with recurrent epistaxis. *J Pediatr* 1988; **113**: 73–6.

16 Higham JM, O'Brien PM, Shaw RW. Assessment of menstrual blood loss using a pictorial chart. *Br J Obstet Gynaecol* 1990; **97**: 734–9.

17 Buchanan GR, Adix L. Grading of hemorrhage in children with idiopathic thrombocytopenic purpura. *J Pediatr* 2002; **141**: 683–8.

18 Nishino M, Girma JP, Rothschild C, *et al*. New variant of von Willebrand disease with defective binding to factor VIII. *Blood* 1989; **74**: 1591–9.

19 Mazurier C, Meyer D. Factor VIII binding assay of von Willebrand factor and the diagnosis of type 2N von Willebrand disease—Results of an international survey. On behalf of the Subcommittee on von Willebrand Factor of the Scientific and Standardization Committee of the ISTH. *Thromb Haemost* 1996; **76**: 270–4.

20 Schneppenheim R, Budde U, Krey S, *et al*. Results of a screening for von Willebrand disease type 2N in patients with suspected haemophilia A or von Willebrand disease type 1. *Thromb Haemost* 1996; **76**: 598–602.

21 Peretz H, Mulai A, Usher S, *et al*. The two common mutations causing factor XI deficiency in Jews stem from distinct founders: One of ancient Middle Eastern origin and another of more recent European origin. *Blood* 1997; **90**: 2654–9.

22 Kanaji T, Okamura T, Osaki K, *et al*. A common genetic polymorphism (46 C to T substitution) in the 5′-untranslated region of the coagulation factor XII gene is associated with low translation efficiency and decrease in plasma factor XII level. *Blood* 1998; **91**: 2010–14.

23 Gordon EM, Donaldson VH, Saito H, Su E, Ratnoff OD. Reduced titers of Hageman factor (Factor XII) in Orientals. *Ann Intern Med* 2001; **95**: 697.

24 Seri M, Pecci A, Di Bari F, *et al*. MYH9-related disease: May–Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome are not distinct entities but represent a variable expression of a single illness. *Medicine (Baltimore)* 2003; **82**: 203–15.

25 Harrison P. The role of PFA-100 testing in the investigation and management of haemostatic defects in children and adults. *Br J Haematol* 2005; **130**: 3–10.

26 Hanseler E, Fehr J, Keller H. Estimation of the lower limits of manual and automated platelet counting. *Am J Clin Pathol* 1996; **105**: 782–7.

27 Bizzaro N. EDTA-dependent pseudothrombocytopenia: A clinical and epidemiological study of 112 cases, with 10-year follow-up. *Am J Hematol* 1995; **50**: 103–9.

28 Giles C. The platelet count and mean platelet volume. *Br J Haematol* 1981; **48**: 31–7.

29 Gohda F, Uchiumi H, Handa H, *et al*. Identification of inherited macrothrombocytopenias based on mean platelet volume among patients diagnosed with idiopathic thrombocytopenia. *Thromb Res* 2007; **119**: 741–6.

30 Racchi O, Rapezzi D. Megathrombocytes and spurious thrombocytopenia. *Eur J Haematol* 2001; **66**: 140–1.

31 Balduini CL. Giant platelet syndromes and the MYH9 mutations. *Lab Hematol* 2004; **10**: 187–8.

32 Andrew M, Castle V, Mitchell L, Paes B. Modified bleeding time in the infant. *Am J Hematol* 1989; **30**: 190–1.

33 Rodgers RP, Levin J. A critical reappraisal of the bleeding time. *Semin Thromb Haemost* 1990; **16**: 1–20.

34 Jilma B. Platelet function analyzer (PFA-100): A tool to quantify congenital or acquired platelet dysfunction. *J Lab Clin Med* 2001; **138**: 152–63.

35 Carcao MD, Blanchette VS, Dean JA, *et al*. The Platelet Function Analyzer (PFA-100): A novel in-vitro system for evaluation of primary hemostasis in children. *Br J Haematol* 1998; **101**: 70–3.

36 Favaloro EJ. Clinical application of the PFA-100. *Curr Opin Hematol* 2002; **9**: 407–15.

37 Quiroga T, Goycoolea M, Muñoz B, *et al*. Template bleeding time and PFA-100 have low sensitivity to screen patients with hereditary mucocutaneous hemorrhages: Comparative study in 148 patients. *J Thromb Haemost* 2004; **2**: 892–8.

38 Hayward CP, Harrison P, Cattaneo M, *et al*. The Platelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Platelet function analyzer (PFA)-100 closure time in the evaluation of platelet disorders and platelet function. *J Thromb Haemost* 2006; **4**: 312–19.

39 Shah MD, O'Riordan MA, Alexander SW. Evaluation of prolonged aPTT values in the pediatric population. *Clin Pediatr* 2006; **45**: 347–53.

40 Humphries JE, Acker MN, Pinkston JE, Ruddy S. Transient lupus anticoagulant associated with prothrombin deficiency: Unusual cause of bleeding in a 5-year-old girl. *Am J Pediatr Hematol Oncol* 1994; **16**: 372–6.

41 Endler G, Marsik C, Jilma B, *et al*. Evidence of a U-shaped association between factor XII activity and overall survival. *J Thromb Haemost* 2007; **5**: 1143–8.

42 Cumming AM, Jones GR, Wensley RT, Cundall RB. In vitro neutralization of heparin in plasma prior to the activated partial thromboplastin time test: An assessment of four heparin antagonists and two anion exchange resins. *Thromb Res* 1986; **41**: 43–56.

43 Funk C, Gmur J, Herold R, Straub PW. Reptilase-R—A new reagent in blood coagulation. *Br J Haematol* 1971; **21**: 43–52.

44 Thompson AR, Counts RB. Removal of heparin and protamine from plasma. *J Lab Clin Med* 1976; **88**: 922–9.

45 Sadler JE, Budde U, Eikenboom JC, *et al*.; Working Party on von Willebrand Disease Classification. Update on the pathophysiology and classification of von Willebrand disease: A report of the Subcommittee on von Willebrand Factor. *J Thromb Haemost* 2006; **4**: 2103–14.

46 Lillicrap D, Nair SC, Srivastava A, *et al*. Laboratory issues in bleeding disorders. *Haemophilia* 2006; **12**(Suppl. 3): 68–75.

47 Gill JC, Endres-Brooks J, Bauer PJ, *et al*. The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood* 1987; **69**: 1691–5.

48 Miller JL, Castella A. Platelet-type von Willebrand's disease: Characterization of a new bleeding disorder. *Blood* 1982; **60**: 790–4.

49 Weiss HJ, Meyer D, Rabinowitz R, *et al*. Pseudo-von Willebrand's disease. An intrinsic platelet defect with aggregation by unmodified human factor VIII/von Willebrand factor and enhanced adsorption of its high-molecular-weight multimers. *N Engl J Med* 1982; **306**: 326–33.
50 Favaloro EJ. Laboratory assessment as a critical component of the appropriate diagnosis and sub-classification of von Willebrand's disease. *Blood Rev* 1999; **13**: 185–204.
51 Cattaneo M. Inherited platelet-based bleeding disorders. *J Thromb Haemost* 2003; **1**: 1628–36.
52 Nurden P, Nurden A. Congenital disorders associated with platelet dysfunctions. *Thromb Haemost* 2008; **99**: 253–63.
53 Cines DB, Blanchette VS. Immune thrombocytopenic purpura. *N Engl J Med* 2002; **346**: 995–1008.
54 Hayward CP, Rao AK, Cattaneo M. Congenital platelet disorders: Overview of their mechanisms, diagnostic evaluation and treatment. *Haemophilia* 2006; **12**(Suppl. 3): 128–36.
55 Hayward CP. Diagnostic approach to platelet function disorders. *Transfus Apher Sci* 2008; **38**: 65–76.
56 Witkop CJ, Krumwiede M, Sedano H, White JG. Reliability of absent platelet dense bodies as a diagnostic criterion for Hermansky–Pudlak syndrome. *Am J Hematol* 1987; **26**: 305–11.
57 Israels SJ, McNicol A, Robertson C, Gerrard JM. Platelet storage pool deficiency: Diagnosis in patients with prolonged bleeding times and normal platelet aggregation. *Br J Haematol* 1990; **75**: 118–21.
58 Kunishima S, Matsushita T, Kojima T, *et al*. Immunofluorescence analysis of neutrophil nonmuscle myosin heavy chain-A in MYH9 disorders: Association of subcellular localization with MYH9 mutations. *Lab Invest* 2003; **83**: 115–22.
59 Balduini CL, Iolascon A, Savoia A. Inherited thrombocytopenias: From genes to therapy. *Haematologica* 2002; **87**: 860–80.

19 Care of the child with hemophilia

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The goal of treatment of a child with hemophilia should be to ensure that both the family and the affected child perceive themselves as healthy, despite the diagnosis of hemophilia. The World Health Organization (WHO) defines health as a state of complete physical, psychological, and social well-being. The aim of this chapter is to consider various aspects of the medical and psychosocial care of children with hemophilia.

Medical care

Diagnosis and risk of inhibitor development

Healthcare professionals are highly aware that hemophilia is a hereditary disorder. However, in many countries today, it is not as well recognized that the majority of babies born with hemophilia actually represent sporadic cases, i.e., the families in question have no known history of hemophilia. This lack of knowledge is a major cause of delay in diagnosis of the disorder, since physicians simply overlook the risk of hemophilia in previously unaffected families.

Once a definite diagnosis has been made based on the results of clotting assays, it is recommended that the mutation in a family be characterized. Knowledge of the type of mutation enables carrier detection and prenatal diagnosis in the family as well as the risk of developing inhibitors directed against factor VIII (FVIII) or factor IX (FIX) [1–3]. The development of inhibitors is today the most serious complication of treatment. Other host-related factors important for inhibitor development, besides the type of mutation and thus the severity of the disease, are family history of inhibitors, ethnicity, and alleles of immune response modifier genes [4–7]. Conflicting results have been obtained if age at first treatment is a risk factor for inhibitor development, i.e., increased risk below the age of 1–1.5 years [8–10]. However, subsequent studies suggested that children who were treated at an early age were treated because of specific circumstances and those circumstances rather than age accounted for the development of inhibitors [11]. Surgical procedures, treatment during concomitant inflammatory states and peak treatment moments, i.e., frequent and extensive administration of high doses of factor concentrate at start of treatment, have been suggested to increase the risk of inhibitor development and should thus be avoided [11,12]. Another aspect of early treatment being currently discussed is whether the mode of administration, regular prophylactic treatment or on-demand, has an impact on inhibitor development [10,12]. The impact of the type of concentrate has also been debated [13,14]. Some papers show that the presence of von Willebrand factor (VWF) in some plasma-derived FVIII products may provide some measure of protection against inhibitor development, although the evidence is not conclusive. Nevertheless, administration of FVIII/FIX concentrates as treatment for a bleed should not be avoided, even if a patient is at risk of developing inhibitors. A child with hemophilia B as a result of a complete gene deletion runs a substantial risk of an anaphylactoid reaction to FIX infusions [15]. Such a response usually occurs after one of the first 10 to 20 infusions, therefore it is recommended that healthcare professionals who give this type of treatment are also prepared to deal with an allergic reaction.

Treatment

The most important aspect of the care of children with hemophilia is the treatment regimen that is used, which varies considerably between countries owing to differences in the level of healthcare that is generally available [16]. The quality of FVIII or FIX replacement therapy in a country usually evolves from sporadic or on-demand treatment of bleeding episodes to secondary prophylaxis for those with frequent bleeds, and finally to individually tailored primary prophylaxis for all children with severe or moderate hemophilia [17,18]. According to a joint statement made by the WHO and the World Federation of Hemophilia (WFH), initiating prophylactic treatment at an early age is considered to be the optimal form of therapy for a child with hemophilia [19]. Today, the most refined regimens involve primary prophylaxis, in which treatment is begun at 12–18 months of age, before the onset of bleeding into joints or other serious bleeds. The rationale behind an early start is that even a small number of joint bleeds can result in irreversible damage, as well as damage that progresses despite prophylactic therapy [20]. It has also been shown that the time point at which prophylaxis is begun is an independent factor in the evaluation of joint outcome [21]. However, it must not be forgotten that the aim

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of prophylactic treatment is to avoid not only arthropathy but also other serious bleedings such as intracranial hemorrhage [22]. In most cases, an early therapeutic approach is initiated by giving a dose of approximately 30–50 IU/kg once or twice a week via a peripheral vein, with the aim of increasing the frequency of administration as soon as possible. The ultimate goal is to reach full-scale primary prophylaxis, which usually involves the following: in hemophilia A, factor VIII is administered at a dose of 20–40 IU/kg/day every second day or three times weekly; in hemophilia B, factor IX is given at a dose of 20–40 U/kg/day every third day or twice weekly [18,23]. However, both the dose and the dose interval have to be individually tailored for each child owing to pharmacokinetic differences between patients. In older children with hemophilia A it is possible to optimize the cost–benefit ratio of treatment by daily injections of FVIII (10–20 IU/kg). The level of the lowest concentration is more important than the peak level after injection [24]. However, it is the clinical outcome, not the achieved trough levels, that determines whether the given dose is adequate [25]. The sizes of the vials available is also a factor that in practice influences the dose given, especially in small children. From both a medical and a social perspective, it is best if the children can be treated at home by their parents, and that particular objective has already been accomplished for most of those patients in countries that have a well-developed system of care for people with hemophilia.

Monitoring treatment

To ensure high quality of care, children with severe hemophilia should be examined once or twice a year by a pediatrician at a comprehensive hemophilia care center. The same applies to children with mild hemophilia, although the check-ups can be done less frequently in such cases. The basic items that are recommended to be included in a biannual or annual check-up are listed in Table 19.1. Joint evaluation should be done once a year by use of a sensitive score such as the Hemophilia Joint Health Score (HJHS) 2.0 [26]. Scoring of bone changes on plain X-ray is still a valuable method and, in addition, magnetic resonance imaging (MRI) can reveal the early changes in synovia and cartilage as an aid in monitoring treatment [27–30]. Quality of life can be evaluated by "Hemoqol," a disease-specific instrument validated for use in children [31]. In countries with less advanced therapy, functional tests may be a valuable tool [32,33].

Table 19.1 Some items that are recommended to be considered at biannual/annual check-ups of children with hemophilia.

Physical examination, including orthopedic joint score
Feedback on "daily log-book" or similar registration of bleeds and treatments
Education of the child and/or parents in venous access
Surveillance of central venous lines (position of catheter, rtPA/urokinase installation in catheter, blood culture, education of the child and/or parents in aseptic techniques)
Laboratory surveillance including blood counts, FVIII/IX levels 24 or 48 h after treatment (when on prophylaxis), FVIII/IX peak values after infusion of prescribed dose, inhibitor analysis
Sociomedical aspects (quality of life, leisure activities, absence from school)

Venous access

Easy venous access is a prerequisite of administering blood factor concentrates to young children with hemophilia A or B, regardless of whether this is done at the time of a bleed or as a prophylactic measure. For a child receiving on-demand treatment at home, it is preferable that the FVIII/FIX concentrate be given by the parents as soon as a bleed occurs. In such a situation, safe and easy access to a vein is essential, and the same is true for children on a prophylactic regimen. The first choice of access should be a peripheral vein. However, that can be very difficult or even impossible to accomplish in young children, thus it may be necessary to consider a central venous line (Figure 19.1). Introduction of a central venous catheter entails risks that must be weighed against the potential benefits for individual patients. Medical indications may include poor access to a peripheral vein for a planned therapy, especially the daily injections that are required for immune tolerance induction (ITI) in patients with inhibitors. An example of a combined medical and social indication is when a central venous line would enable parents to treat a young child at home. Implantation of a central venous catheter solely on psychological grounds should be discouraged—a child who is merely afraid of venipuncture needs to be helped in some other way.

Several reports have described various adverse effects associated with the use of central venous catheters in patients with

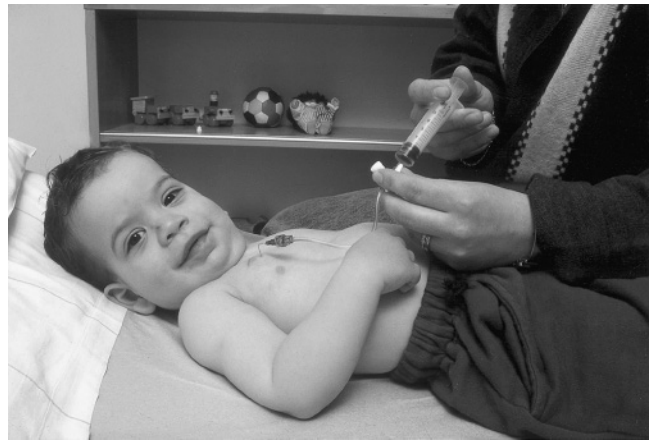

Figure 19.1 An implantable central venous line facilitates injections in children with difficult venous access in peripheral veins.

Table 19.2 The rates of infections in recent series of hemophilia patients using central venous lines.

Study	Number of patients (*n*)	Rate of infection per 1000 patient-days	Comment
Blanchette *et al.* [41]	19	0.7	Three patients with inhibitors, three HIV positive
Perkins *et al.* [42]	35	1.2 (central device) 0.7 (peripheral device)	7/32 patients with inhibitors, 2/32 with von Willebrand disease
Ljung *et al.* [43]	53	0.19	Multicenter. Eleven patients with inhibitors
Santagostino *et al.*[44]	15	0.3	Two patients with inhibitors, 13 on prophylaxis; prospective
Miller *et al.* [45]	41	0.14	Includes external catheters
McMahon *et al.* [46]	58	1.6 (without inhibitor) 4.3 (with inhibitor)	77/86 had Port-A-Caths; 37/58 patients with hemophilia
Tarantino *et al.*[47]	59	0.38 (without inhibitor) 0.66 (with inhibitor)	Single institution
Van Dijk *et al.* [48]	23	0.72 (without inhibitor) 3.1 (with inhibitor)	Single institution
Domm *et al.* [49]	22	0.30	Single institution; 9/22 patients with inhibitors

hemophilia, and infections were the most frequently mentioned complications in those subjects [34]. Table 19.2 shows the rates of infections that have been observed in some of the larger studies conducted in recent years. It appears that non-inhibitor patients fall into two major categories: Those with approximately 0.2 infections per 1000 days, and those with roughly 1.0 (range 0.7–1.6) infections per 1000 days. In the best of hands, a patient without inhibitors who has a Port-A-Cath and is on regular prophylaxis will have at most one catheter-related infection every 5–10 years, although this rate varies greatly between different centers. In an equivalent patient who has developed inhibitors, it can be expected that there will be about one infection per 1–2 years of use [35]. Notwithstanding, easy venous access is imperative for these patients, both for the treatment of acute bleeds and for ITI.

The rates of clinically manifest thrombosis have been low in the larger series of patients that have been documented, but it should be noted that routine venography was not done in most of those series. Recent reports suggest that thrombosis, although in most instances "clinically silent," is a more frequent side-effect than previously assumed [34,36]. The experiences so far suggest that the risk of catheter-related thrombosis increases after many years of use. Development of thrombosis may be related to the site of the catheter (jugular or subclavian vein), the type of concentrate used, or some genetic thrombophilic factor [37].

The final decision to use a central venous catheter must be a compromise between the following: the medical goal, the bleeding tendency and the social situation of the patient, and familiarity with the devices at the particular hemophilia center. The number of complications may be reduced by taking adequate measures to maintain asepsis, both at the time of implantation and during subsequent use, and also by adopting explicit basic routines for surveillance of the systems and repeatedly educating the users [38]. In many cases, a central venous line is indispensable for appropriate treatment, and several series on record have clearly demonstrated the benefits of these devices for hemophilic children and their families. Some centers have successfully used arteriovenous fistulae in children as an alternative to a central venous line [39].

Medication

In developed countries, most children with hemophilia are treated with recombinant FVIII/IX concentrates [40]. Drugs containing acetylsalicylic acid should not be used to relieve pain, because they inhibit platelet function and therefore have an adverse effect on coagulation. Consequently, preparations containing acetaminophen (paracetamol), alone or in combination with codeine, are recommended as analgesics. Anti-inflammatory drugs, such as celecoxib and rofecoxib, can in certain cases be useful in reducing joint pain and synovial inflammation.

Psychological care

In countries with limited resources for medical care, it is natural to focus on hemophilia itself and literally on how to help patients survive from day to day. In most countries with well-developed health care, the ability to treat this disease has improved dramatically during the last decades owing to the introduction of FVIII and FIX concentrates. Therefore, in those nations, the focus should be switched from the disorder per se to the healthy aspects of the child with hemophilia. The connotation of the word "hemophilia" and the description of the condition have a markedly negative influence on how it is perceived by the parents and later on by the child. This disease has a dramatic history, and the attitudes of healthcare professionals and older people with hemophilia are still affected by the way the disorder used to be managed, even in industrialized countries.

The initial information given to a family with a child who has been diagnosed with hemophilia will have a pronounced effect on how this family, and in time the child himself, will cope with the disease and how it will influence daily life. Ideally, this information should be given to both parents, if possible together with older siblings. If advanced hemophilia care is available, the most important message to convey in the first discussion is that a person with hemophilia can lead a practically normal life and have a normal life expectancy. It is of the essence that children who are old enough to understand the situation do not feel that, by not being healthy, they have caused problems for their parents and made them unhappy. A young child lives in the here and now, whereas the parents have a totally different time perspective and are more extensively influenced by existential thoughts. The mother might be a genetic carrier, and thus it is obvious that she may feel responsible for her child's hemophilia.

For an optimal outcome of the crisis reaction, it is important to try to discover whether the parents think that they are to blame or that they have done something wrong in the past that might have given rise to the disease.

The initial counseling should be repeated at subsequent meetings with the parents to ensure that they have received all relevant information. Positive facts about prophylactic treatment strategies and the prospect of being able to use gene therapy to cure the disease in the future should be given together with straightforward information about possible complications, such as the development of inhibitors. Furthermore, it is important that the same doctor and nurse communicate with the family during this sensitive period in order to avoid the uncertainty and frustration that can result from slight differences in the way that individual care providers present the same information.

The child suffering from hemophilia is not the only person who is influenced by the disease, there is also a profound psychosocial effect on all members of the family. Overprotection may become a serious problem, and the strong natural urge to safeguard the child can instead cause difficulties for the entire family. Indeed, in extreme cases, the misguided love of the parents may be more harmful to a son with hemophilia than the disorder itself. Therefore, it is essential that the pediatric hemophilia team work together with the families to support and promote normal behavior.

Social care

Hemophilia identification cards

In conjunction with the diagnosis, a patient with hemophilia should be issued a card that states the type of the disease that the bearer has, and that also provides information about how to contact the hemophilia treatment center. It is important that the patient always carries the card and shows it when consulting a physician or undergoing dental work.

Vaccinations

Children with hemophilia can be vaccinated like any other children, but the vaccines must be given subcutaneously, not intramuscularly. It is recommended not to give concentrate at the same time as a vaccination as a measure to avoid inhibitor development. Moreover, in countries with low-purity concentrates or cryoprecipitates used for treatment, vaccination against hepatitis A and B is recommended.

Day-care center attendance and school

Attending a day-care center is no problem for a child with hemophilia, although it is recommended that the staff of the facility have access to some extra resources. The family has to avoid overprotection, and day care or other activity groups can provide just the social training and stimulation that the child needs. The school staff and students should be informed that a child has hemophilia, preferably by the parents and the child, if necessary together with the staff of the hemophilia center. If adequate prophylaxis is given, no other resources are required for medical reasons, although it is important to coordinate prophylactic treatment with the scheduling of physical education. Also, the study and vocational counselor should be told about any limitations that the disease imposes on the child's choice of profession.

Leisure activities

A child with hemophilia who is on adequate prophylaxis can enjoy virtually normal free-time activities, but it is best to steer clear of contact sports that involve a high risk of traumatic events. Regardless of the mode of therapy, parents should be encouraged to stimulate the child's interest in certain suitable sports (such as swimming) at an early age. A hemophilic baby can wear a protective cap or helmet from the time it begins to stand up and until it has learned to walk steadily. However, as the child grows older, a helmet may be a social stigma that should be avoided. Parents and other people who take care of a young hemophiliac should be continually encouraged to observe and focus on the healthy side of the child.

References

1 Schwaab R, Brackmann HH, Meyer C, *et al*. Haemophilia A: Mutation type determines risk of inhibitor formation. *Thromb Haemost* 1995; **74**: 1402–6.
2 Giannelli F, Choo KH, Rees DJ, Boyd Y, Rizza CR, Brownlee GG. Gene deletions in patients with haemophilia B and anti-factor IX antibodies. *Nature* 1983; **303**: 181–2.
3 Ljung RC. Gene mutations and inhibitor formation in patients with hemophilia B. *Acta Haematol* 1995; **94**(Suppl. 1): 49–52.
4 Oldenburg J, Pavlova A. Genetic risk factors for inhibitors to factors VIII and IX. *Haemophilia* 2006; **12**(Suppl. 6): 15–22.
5 Astermark J, Oldenburg J, Pavlova A, Berntorp E, Lefvert AK. Polymorphisms in the IL10 but not in the IL1beta and IL4 genes

are associated with inhibitor development in patients with hemophilia A. *Blood* 2006; **107**: 3167–72.
6 Astermark J, Oldenburg J, Carlson J, *et al*. Polymorphisms in the TNFA gene and the risk of inhibitor development in patients with hemophilia A. *Blood* 2006; **108**: 3739–45.
7 Astermark J, Oldenburg J, Escobar M, White GC, 2nd, Berntorp E. The Malmo International Brother Study (MIBS). Genetic defects and inhibitor development in siblings with severe hemophilia A. *Haematologica* 2005; **90**: 924–31.
8 Lorenzo JI, Lopez A, Altisent C, Aznar JA. Incidence of factor VIII inhibitors in severe haemophilia: The importance of patient age. *Br J Haematol* 2001; **113**: 600–3.
9 van der Bom JG, Mauser-Bunschoten EP, Fischer K, van den Berg HM. Age at first treatment and immune tolerance to factor VIII in severe hemophilia. *Thromb Haemost* 2003; **89**: 475–9.
10 Santagostino E, Mancuso ME, Rocino A, *et al*. Environmental risk factors for inhibitor development in children with haemophilia A: A case–control study. *Br J Haematol* 2005; **130**: 422–7.
11 Gouw SC, van den Berg HM, le Cessie S, van der Bom JG. Treatment characteristics and the risk of inhibitor development: A multicenter cohort study among previously untreated patients with severe hemophilia A. *J Thromb Haemost* 2007; **5**: 1383–90.
12 Gouw SC, van der Bom JG, Marijke van den Berg H. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: The CANAL cohort study. *Blood* 2007; **109**: 4648–54.
13 Goudemand J, Laurian Y, Calvez T. Risk of inhibitors in haemophilia and the type of factor replacement. *Curr Opin Hematol* 2006; **13**: 316–22.
14 Gouw SC, van der Bom JG, Auerswald G, Ettinghausen CE, Tedgard U, van den Berg HM. Recombinant versus plasma-derived factor VIII products and the development of inhibitors in previously untreated patients with severe hemophilia A: The CANAL cohort study. *Blood* 2007; **109**: 4693–7.
15 Warrier I, Ewenstein BM, Koerper MA, *et al*. Factor IX inhibitors and anaphylaxis in hemophilia B. *J Pediatr Hematol Oncol* 1997; **19**: 23–7.
16 Aledort L. Unsolved problems in haemophilia. *Haemophilia* 1998; **4**: 341–5.
17 Van Den Berg MDPDHM, Fischer K. Prophylaxis for severe hemophilia: Experience from Europe and the United States. *Semin Thromb Hemost* 2003; **29**: 49–54.
18 Nilsson IM, Berntorp E, Lofqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B [see comments]. *J Intern Med* 1992; **232**: 25–32.
19 Berntorp E, Boulyjenkov V, Brettler D, *et al*. Modern treatment of haemophilia. *Bull World Health Organ* 1995; **73**: 691–701.
20 Kreuz W, Escuriola Ettingshausen C, Funk M, Schmidt H, Kornhuber B. When should prophylactic treatment in patients with haemophilia A and B start?—The German experience. *Haemophilia* 1998; **4**: 413–17.
21 Astermark J, Petrini P, Tengborn L, Schulman S, Ljung R, Berntorp E. Primary prophylaxis in severe hemophilia should be started early but can be individualized. *Br J Haematol* 1999; **105**: 1109–13.
22 Ljung RC. Intracranial haemorrhage in haemophilia A and B. *Br J Haematol* 2008; **140**: 378–84.
23 Ljung RC. Can haemophilic arthropathy be prevented? *Br J Haematol* 1998; **101**: 215–19.
24 Carlsson M, Berntorp E, Bjorkman S, Lindvall K. Pharmacokinetic dosing in prophylactic treatment of hemophilia A. *Eur J Haematol* 1993; **51**: 247–52.
25 Björkman S. Prophylactic dosing of factor VIII and IX from a clinical pharmacokinetic perspective. *Haemophilia* 2003; **9**(Suppl. 1): 101–10.
26 Hilliard P, Funk S, Zourikian N, *et al*. Hemophilia joint health score reliability study. *Haemophilia* 2006; **12**: 518–25.
27 Pettersson H, Ahlberg A, Nilsson IM. A radiologic classification of hemophilic arthropathy. *Clin Orthop* 1980; **149**: 153–9.
28 Manco-Johnson M, Nuss R, Funk S, Murphy J. Joint evaluation instruments for children and adults. *Haemophilia* 2000; **6**: 649–57.
29 Kilcoyne R, Nuss R. Radiological assessment of haemophilic arthropathy with emphasis on MRI findings. *Haemophilia* 2003; **9**(Suppl. 1): 57–64.
30 Lundin B, Babyn P, Doria AS, *et al*. Compatible scales for progressive and additive MRI assessments of haemophilic arthropathy. *Haemophilia* 2005; **11**: 109–15.
31 Bullinger M, Von Mackensen S. Quality of life in children and families with bleeding disorders. *J Pediatr Hematol Oncol* 2003; **25**(Suppl. 1): S64–7.
32 Poonnoose PM, Manigandan C, Thomas R, *et al*. Functional Independence Score in Haemophilia: A new performance-based instrument to measure disability. *Haemophilia* 2005; **11**: 598–602.
33 van Genderen FR, van Meeteren NL, van der Bom JG, *et al*. Functional consequences of haemophilia in adults: The development of the Haemophilia Activities List. *Haemophilia* 2004; **10**: 565–71.
34 Ljung R. The risk associated with indwelling catheters in children with haemophilia. *Br J Haematol* 2007; **138**: 580–6.
35 Ljung R, van den Berg M, Petrini P, *et al*. Port-A-Cath usage in children with haemophilia: Experience of 53 cases. *Acta Paediatr* 1998; **87**: 1051–4.
36 Price VE, Carcao M, Connolly B, *et al*. A prospective, longitudinal study of central venous catheter-related deep venous thrombosis in boys with hemophilia. *J Thromb Haemost* 2004; **2**: 737–42.
37 Ettingshausen C, Kurnik K, Schobess R, *et al*. Catheter related thrombosis in children with haemophilia A. Evidence of a multifactorial disease. *Blood* 2002; **99**: 449–500.
38 Ljung R. Central venous lines in haemophilia. *Haemophilia* 2003; **9**(Suppl. 1): 88–93.
39 Santagostino E, Gringeri A, Berardinelli L, Beretta C, Muca-Perja M, Mannucci PM. Long-term safety and feasibility of arteriovenous fistulae as vascular accesses in children with haemophilia: A prospective study. *Br J Haematol* 2003; **123**: 502–6.
40 Chambost H, Ljung R. Changing pattern of care of boys with haemophilia in western European centres. *Haemophilia* 2005; **11**: 92–9.
41 Blanchette VS, al-Musa A, Stain AM, Filler RM, Ingram J. Central venous access catheters in children with haemophilia. *Blood Coagul Fibrinolysis* 1996; **7**(Suppl. 1): S39–44.
42 Perkins JL, Johnson VA, Osip JM, *et al*. The use of implantable venous access devices (IVADs) in children with hemophilia. *J Pediatr Hematol Oncol* 1997; **19**: 339–44.
43 Ljung R, Petrini P, Lindgren AK, Berntorp E. Implantable central venous catheter facilitates prophylactic treatment in children with haemophilia. *Acta Paediatr* 1992; **81**: 918–20.
44 Santagostino E, Gringeri A, Muca-Perja M, Mannucci PM. A prospective clinical trial of implantable central venous access in children with haemophilia. *Br J Haematol* 1998; **102**: 1224–8.
45 Miller K, Buchanan GR, Zappa S, *et al*. Implantable venous access devices in children with hemophilia: A report of low infection rates [see comments]. *J Pediatr* 1998; **132**: 934–8.

46 McMahon C, Smith J, Khair K, Liesner R, Hann I, Smith O. Central venous access devices in children with congenital coagulation disorders; complications and long-term outcome. *Br J Haematol* 2000; **110**: 461–8.
47 Tarantino MD, Lail A, Donfield SM, *et al*. Surveillance of infectious complications associated with central venous access devices in children with haemophilia. *Haemophilia* 2003; **9**: 588–92.
48 Van Dijk K, Van Der Bom JG, Bax KN, Van Der Zee DC, Van Den Berg MH. Use of implantable venous access devices in children with severe hemophilia: Benefits and burden. *Haematologica* 2004; **89**: 189–94.
49 Domm JA, Hudson MG, Janco RL. Complications of central venous access devices in paediatric haemophilia patients. *Haemophilia* 2003; **9**: 50–6.

20 Adolescence

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One may estimate that of the 100 000 adolescents with hemophilia worldwide a great majority do not have access to modern treatment possibilities, resulting in elevated morbidity and mortality.

A study on therapeutic regimen patterns in boys treated for severe hemophilia in 22 European centers in 2003 indicated that almost all patients were on different prophylactic treatments [1].

Progression of physical development at puberty

The psychosocial maturation of adolescents is not possible without the onset of puberty. A boy may resist change. He may be unable to deal with *a new mature sexual body* and *a new self-image*. This may result in discrepancy between the imaginary and the real self.

In an average boy, puberty starts almost 2 years later than in an average girl, and the maximum growth spurt in body height starts even later, at mid-puberty, which is 3–3.5 years later than in girls. The developmental differences between boys and girls are greatest at the time when an average boy is in mid-puberty, around the age of 13.5 years in most European populations. Even though there are remarkable individual variations in timing, the sequence and duration of pubertal events tend to progress in a certain order.

First, the testicles grow in size. Once the testicles reach a length of about 2 cm, pubic hair starts to develop. The mean growth period for testicles is relatively long, about 4 years, whereas the growth of penis starts about a year later and reaches its final size relatively quickly, in about 2 years (Figure 20.1). By mid-puberty the penis is growing fast and has almost reached its final size. The voice is changing, nocturnal spontaneous ejaculations ("wet dreams") have started, regular masturbation is a part of life, gynecomastia may disturb many boys, spontaneous erections are uncontrolled, and acne may gradually appear. By the time axillary hair appears, the hands, feet, and penis will already have reached their final sizes. By the end of puberty, the body growth in height and weight starts to reach its maximum.

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A delayed beginning of puberty is a male phenomenon. It is known that late maturers often have a low self-esteem and that feeling of inadequacy is common. They are seen by their peers as being more childish and are less popular than the average boy.

Almost nothing is known about the potential consequences of rapidly passing puberty. However, if the pubertal period is short, it is likely that the *psychological adaptation* will be more demanding.

In boys with hemophilia, pubertal developments start and progress similarly as in other boys.

Adolescents with hemophilia should be physically fit

Physical activity helps to maintain a normal body weight, a higher level of self-esteem, body image, social adaptation, and feelings of well-being. Trained muscles may give some protection against injuries. A boy with a nimble and swift-moving body can also avoid physical problems.

The family attitudes, particularly those of the father, are important in developing interest in sports and normal fitness activities. The interest of the care-giver, the hemophilia team, and its physiotherapist may also have a critical role in modifying the parents' motivation during the years before puberty.

There are several approaches to selecting a suitable physical activity. This author emphasizes that restriction is not the only possibility. In fact, most adolescent boys who have been on primary prophylaxis from an early age and continue this through adolescence can participate in almost any sport they wish. Furthermore, they do it well. Under optimal conditions physical activity and the social surroundings in a team should be combined and may be of great value for social development and masculine self-esteem.

In general, it is not an easy task for the hemophilia treater to gradually convince the parents to change their focus from protective attitudes to enjoyment of physical well-being. Nevertheless, this may not be possible without accepting the risks involved.

A pubertal boy can be really stubborn if he wants, for example, to play ice-hockey. In many cases it may be easier for a boy to attend an ice-hockey session or two, and as a result get some painful bruising. Then he himself can make his own open or silent decision that ice-hockey is not for him.

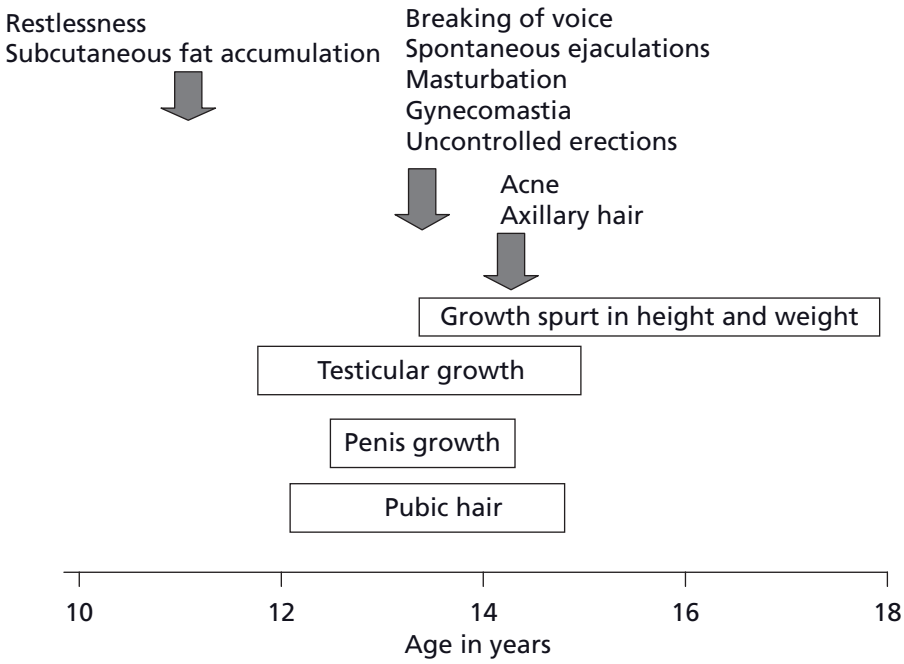

Figure 20.1 Physiologic events in pubertal boys. These events are not influenced by hemophilia per se but are similar in boys with hemophilia [2].

To do so, he may need a suitable cover-up story to maintain his self-esteem.

Shoes should be of high quality

It is common for the ankles of adolescents to ache after repeated bleeds or without any obvious reason. Adhering to the practice of wearing large tennis shoes without laces may not be the correct response. In fact, high-quality shoes should be recommended for adolescents with hemophilia to give optimal and personal support during activities. Shoes should be light and flexible with a good grip and an absorbing sole. On the other hand, whenever possible, wearing no shoes at all might be ideal.

Overweight is a risk factor that can be prevented

Adolescents with hemophilia should not be overweight because any extra weight is an additional burden on the joints of lower extremities. A 10-kg excess weight in the body may easily double the joint stress caused by running, jumping, or similar activities.

Even though it is not easy to prevent becoming overweight, it is much easier to prevent than to treat. For this reason the hemophilia teams might consider starting discussions on weight control soon after the diagnosis of hemophilia as a routine part of the therapy. Those on prophylactic treatment are regularly and frequently seen by their physician and the supporting hemophilia team. This long-term follow-up period should give enough time to create the motivation for a change in the family's practices and allow the care-giver to offer continual support.

The years of prepuberty as a risk period

Several changes occur in the male body in the first 2 years prior to the onset of puberty. For example, subcutaneous fat is accumulated primarily in the lower extremities. This, together with an increase in muscle mass, results in a substantial rise in the weight of the lower extremities. Boys get "a new pair of limbs," which may not function as they used to, leading to clumsiness. The phenomenon may be associated with the increased risk of joint bleeds seen during this period. In fact, many boys on prophylaxis seem to have a period without any major bleeding problems over years 5–9 followed by this period where repeated joint and muscle bleeds occur.

The general restlessness associated with the years of prepuberty may also cause minor accidents when the control of the whole body is not optimal.

Should boys with hemophilia on prophylaxis be independent in the years prior to the onset of puberty?

Boys tend to be exceptionally social and open-minded a couple of years before puberty at about the age of 10–12 years. This

is probably the optimal age to learn more about hemophilia and refill potential gaps with new information, including the techniques of self-injections and personal administration of the clotting factors, if not learned earlier. It would also mean that a new skill has been adopted as a personal routine by the time of the onset of puberty. Boys at this age tend to appreciate mechanical challenges, too.

However, the process of transition from parental dependency, first to partial autonomy, and later to total autonomy, may produce confusion as to who is responsible. Accordingly, the transition age may differ between individuals. Furthermore, the process should not be compulsory. Parental motivation toward their son gaining independence and active support should continue via different means.

The onset of puberty starts the psychosocial development and regression in adolescents

Adolescence is an exceptional developmental phase (Table 20.1). During no other phase do simultaneous forward and backward developments occur with rapid fluctuations between advancement and regression. This phenomenon culminates at mid-puberty.

Regression is necessary for normal adolescent development (Table 20.2). Regression occurs mainly in the areas of psychosexual and psychosocial development. It is a normal series of events, which allow a boy to relieve his earlier childish world of experiences and gradually become separated from these. Regression is a passing phase, a stepping stone on the path to autonomy.

In contrast, cognitive skills develop further regardless of psychosocial regression (Figure 20.2). Progress in personal growth and development is satisfying in particular if it occurs simultaneously with that of close peer. The adolescent goes through three phases during his maturation: (i) He separates himself from his parents; (ii) he takes control of his new and different-looking body; and (iii) he turns to his peers for help in dealing with his growth. During this developmental process the boy needs stability. For example, it might be unjustified to make changes in his hemophilia care.

Developmental regression is crucially important in the maturation of the adolescent male.

Table 20.1 The developmental tasks of adolescence. Many of these developmental tasks may have an influence on hemophilia care in pubertal boys with hemophilia on prophylaxis [2].

Emotional separation from parents
A second chance to change the personality in a crucial and final way
A time period of possibilities
The disengagement from infantile objects and the establishment of a new relationship with parents and other adults in the community
Achievement of biological and sexual maturation
Use of peers in personal growth
Establishment of autonomy

Overprotection from parents

Any chronic disease of childhood may result in changes in the relationships between the son and his parents.

A common trend is that the link between the son and his mother is physiologically strong and continues to strengthen, whereas that between the son and his father is weak and weakens further (Figure 20.3). A mother may have feelings of guilt because hemophilia was inherited from her family. Overprotection does not support the optimal psychosocial maturation of a boy with hemophilia, and may disturb the psychological separation process from the parents, which is necessary during adolescence. It may also weaken the above-mentioned regression, which does not support normal development either. Even if the hemophilia treater is right and the boy's development would benefit from discussing the matter, it may be a demanding approach. Furthermore, it is not always possible to bring up the matter in discussions unless the physician knows the family and their relationships well. We encourage, however, open discussions since they benefit the patient.

We prefer that the father, rather than the mother, attend the routine visits to the adolescent hemophilia center. Only half of adolescents live with both their biologic parents. This is an additional burden in the discussions of family relationships, which must be taken into account.

Table 20.2 Psychosocial regression is a normal phenomenon and necessary for normal adolescent development. Many of these regression-related changes may have an influence on hemophilia care in pubertal boys with hemophilia on prophylaxis [2].

Child-like features are again emphasized
Fluctuating relationship with parents varies from close to distant
Compulsive features are emphasized
Stubbornness increases
Bad manners are the norm; untidiness, breaking wind, swallowing in one gulp, and gorging food
The use of language changes
Impoverishment of spoken language
Written sentences become shorter
Regular use of swear words
Lower academic performance at school
Difficulties concentrating
Motivation to learn is decreasing
Consequences at home
Loosening of parental ties
Communication with adults loses its value
Revolt against the parents

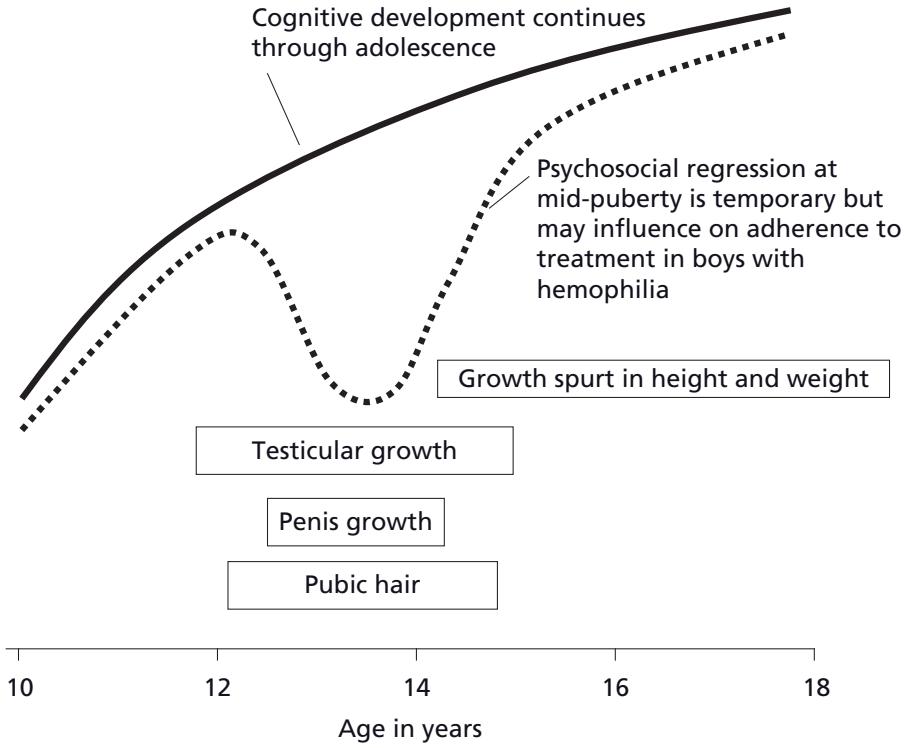

Figure 20.2 The schematic representation illustrates the continuous development of mind in an average adolescent boy. The solid line represents the development of his cognitive abilities and the broken line the maturation of his psychosocial means (psychosocial regression). The regression (Table 20.2) is necessary for a normal development but it should be temporary and culminate at mid-puberty. Adhere to self-administered treatments may be lost in boys with hemophilia during the regression period, which may cause unnecessary joint bleeds [2].

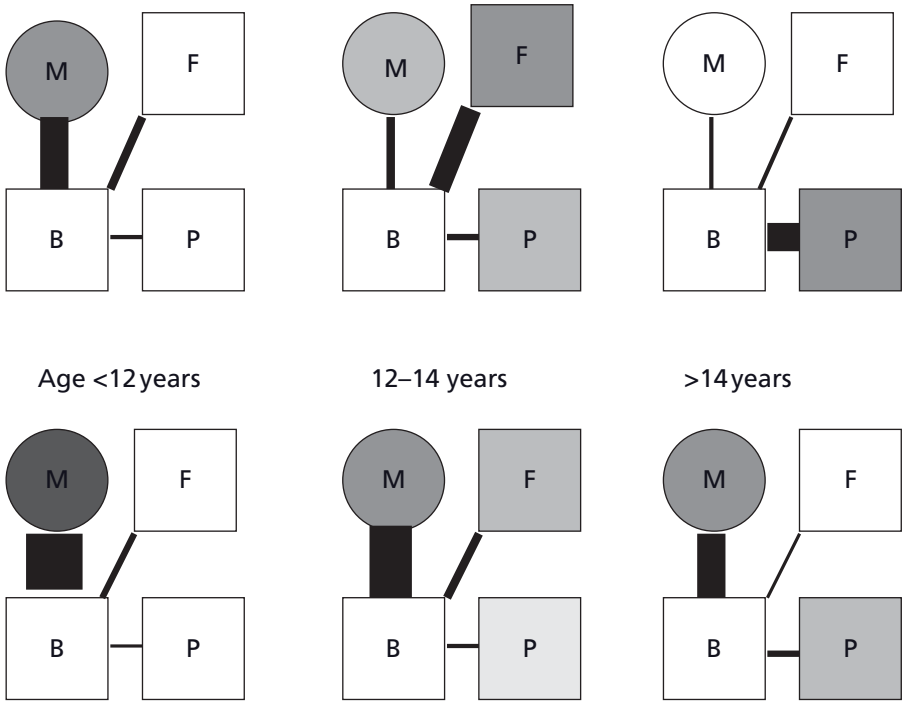

Figure 20.3 Schematic representation of the relationship between the mother (M), father (F), peer (P), and the son (B) during optimal male development (above). There is an increased risk that the relationship between the mother and her son with hemophilia remains too close and disturbs the development of other relationships (below) [2].

Adherence to clotting factor treatment during pubertal psychosocial regression

The adherence to self-administered therapy in adolescent patients is not always complete. The specific deficiencies in the adherence to regular self-administered clotting factor therapy among adolescents with hemophilia are not well defined. The consequences, however, may be immediate with the occurrence of joint bleeds, which have been prevented for years with regular injections. As even only a few joint bleeds may result

in joint destruction during the coming years, any unnecessary bleed should be avoided. Knowledge of the normal psychosocial maturation of pubertal male, as discussed earlier, may help us to understand the thinking of the boy.

Start and duration of prophylactic treatment

Most European adolescents with severe hemophilia are on prophylactic regimens [1]. Available evidence from Sweden, the Netherlands, Denmark, and Germany indicates that only an early onset of prophylaxis in boys with severe hemophilia can prevent arthropathy in adolescence. Even a small number of joint bleeds in children may cause irreversible hemophilic joint disease in later years [3,4]. Accordingly, it may be as a result of events that occurred in early childhood.

A relatively small proportion of patients on replacement therapy has thus far continued prophylaxis through their childhood and adolescence. However, this group of patients is rapidly increasing because of the increasing intensity of the treatment over the past 10–15 years. The open questions relate to the duration and type of treatment in their future. Should the prophylaxis be continued through adulthood, individualized or discontinued and replaced by on-demand therapy. Because no objective answers are available, the decisions should be based on the patient's views and lifestyle. Furthermore, the topic is relevant only in the most affluent countries and societies [5–7].

Transition of care to the adult center

Different strategies may be justified and are, in practice, in the transition of hemophilia care from a pediatric unit to the adult center. One may emphasize that early independence is of value itself and that the transition should occur as late as possible [8].

The current situation is such that adolescents are doing so much better than adults in most developed countries. Patients under 20 years of age show hardly any evidence of chronic joint disease, hepatitis B or C, human immunodeficiency virus (HIV), or any other health-related problems caused by hemophilia or its treatment. The distinction between children and adults may also have an influence on the transition.

The transition should include at least one joint meeting with the pediatric and adult team (Table 20.3). Overlapping clinics for a short period may also be beneficial. Our experience also suggests that it must be fixed to avoid the confusion caused by phone calls, communications, and visits in both centers.

Military service

Military service is not considered possible for patients with hemophilia by military forces.

Table 20.3 Key elements of transitional care [8].

An early start—When children enter a pediatric service, they should know they can expect to leave it
A key worker for each individual
A written transition policy between pediatric and adult services
A flexible policy on timing of events
Skills training in communication, decision making, creative problem solving, assertiveness, self-care, self-determination, and self-advocacy
An educational program for patient and parent which addresses medical, psychosocial, and educational/vocational aspects of care
Administrative support, including provision of a medical summary that is portable and accessible
A training program in adolescent health and transition care for pediatric and adult team members
Primary and preventive care involvement and provision
Affordable continuous health insurance coverage (if appropriate) throughout adolescence and adulthood

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This may be an additional burden among adolescents with hemophilia particularly in the countries where a majority of young men attend military service. The situation may influence the youth's self-esteem, which is difficult to compensate for by other means.

References

1 Chambost H, Ljung R. Changing pattern of care of boys with haemophilia in western European countries. *Haemophilia* 2005; **11**: 92–9.

2 Siimes MA, Aalberg V, Petrini P. *Boys with Haemophilia: Physical and Psychosocial Development at and after Puberty*. Helsinki: Nemo Publisher, 2006.

3 Astermark J, van der Bom JG, Tengborn L *et al.* Primary prophylaxis in severe haemophilia should be started at an early age but can be individualized. *Br J Haematol* 1999; **105**: 1109–1113.

4 Kreuz W, Escuriola-Erringshausen C, Funk M *et al.* When should prophylactic treatment in patients with haemophilia A and B start? The German experience. *Haemophilia* 1998; **4**: 413–417.

5 Dijk van K, Fischer K, van der Bom JG *et al.* Can long-term prophylaxis for severe haemophilia be stopped in adulthood? Results from Denmark and the Netherland. *Br J Haematol* 2005; **130**: 107–112.

6 Fischer K, van der Bom JG, Prejs R *et al.* Discontinuation of prophylactic therapy in severe haemophilia: incidence and effects on outcome. *Haemophilia* 2001; **7**: 544–550.

7 Fischer K, van der Bom JG, Mauser-Bunschoten EP *et al.* The effects of postponing prophylactic treatment on long-term outcome in patients with severe haemophilia. *Blood* 2002; **99**: 2337–2341.

8 McDonagh JE, Viner RM. Lost in transition? Between paediatric and adult services. *BMJ* 2006; **332**: 435–6.

21 Hemophilia and medicine in old age

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Introduction

In 2001, life expectancy of patients with hemophilia in industrial countries increased to over 70 years [1,2]. When excluding individuals infected with human immunodeficiency virus (HIV) and hepatitis C virus (HCV), life expectancy of Dutch patients with mild and moderate hemophilia approached that of the male population in general (75 years compared with 76 years), and life expectancy of patients with severe hemophilia increased to 71 years [2]. So hemophilia is no longer a disorder of children and young adolescents. Nowadays, almost half of all patients treated at the van Creveldkliniek, a large hemophilia treatment center for children and adults in the Netherlands, are born before 1967 (Figure 21.1) [3].

Elderly hemophilia patients have different problems compared with the younger generations. They not only have to live with arthropathy, HCV, and/or HIV infection, but a growing number will suffer from comorbidity, such as internal and cardiovascular disease, urologic problems, or cancer. Little has been published on age-related comorbidity in hemophilia and its psychosocial impact. This chapter focuses on age-related comorbidity in the aging hemophilia patient, and its consequences. It is partially based on the book *Aging with Haemophilia—Medical and Psychosocial Impact* [3] and a review on comorbidity in the aging hemophilia patient, which has been published in *Haemophilia* [4].

Comorbidity is defined as the effect of all other diseases that an individual patient might have, other than the primary disease of interest.

Internal diseases

Hypertension

Observational studies from the past have shown that hemophilia patients have a higher mean blood pressure, have twice as often hypertension and use more antihypertensive medication compared with the general population [5–7]. An explanation might be that the incidence of renal insufficiency is higher in patients with hemophilia [8,9]. This may be caused by renal bleeding in the past, HIV infection, or medication such as tranexamic acid or protease inhibitors. Hypertension increases the risk of myocardial infarction, and adequate treatment is obligatory. Furthermore, hypertension increases the risk of intracranial hemorrhage (ICH). The French ICH study group analyzed 123 ICH episodes in patients with hemophilia, and found that 20 (16.3%) of them occurred in patients >50 years [10]. Interestingly, the proportion of patients with severe hemophilia decreased with age (Table 21.1) [10,11], and in 12.5% of the ICH episodes hypertension was found. The presence of hypertension was not associated with an increased risk of death owing to ICH.

Recommendation

Since they are at higher risk of developing hypertension, blood pressure in hemophilia patients should be regularly checked and hypertension adequately treated. In the absence of other cardiovascular risk factors, a systolic blood pressure of ≤140 mmHg and a diastolic pressure of ≤90 mmHg should be pursued. In case of increased risk for cardiovascular disease, such as in the presence of diabetes mellitus or a positive family history for cardiovascular events, a systolic pressure of ≤130 mmHg and a diastolic pressure of ≤80 mmHg should be aimed for.

Renal abnormalities

There are few recent publications on renal abnormalities in hemophilia. In the 1970s it was studied by Prentice and Beck [8,12]. The majority of lesions were seen in the upper renal tract and were apparently the result of clot formation. This may be negatively influenced by the use of tranexamic acid during hematuria. Abnormalities on intravenous pyelography and isotope renography were also found [8]. A more recent publication describes a strong association between hypertension and HIV, and chronic and acute renal disease in hemophilia patients [13]. An association with inhibitors and recent hematuria was described.

Chronic kidney disease is an independent risk factor for cardiovascular disease and is associated with a worse

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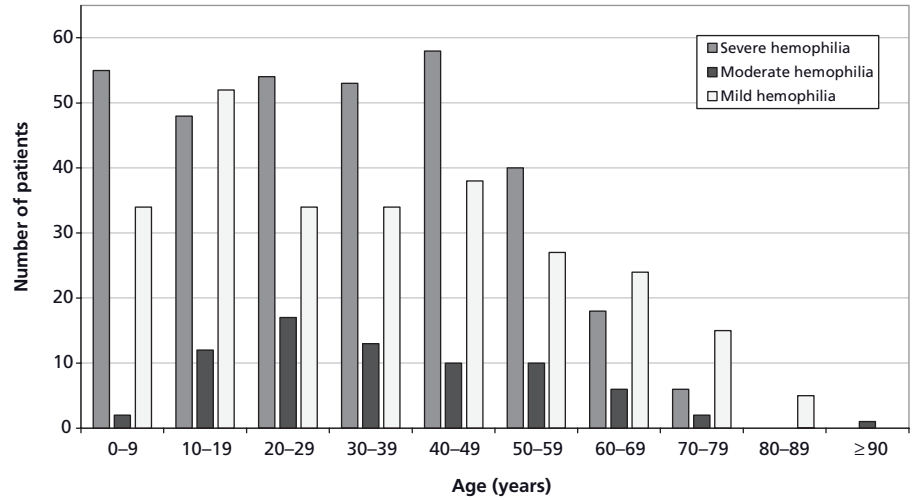

Figure 21.1 Number of patients, according to age and severity of hemophilia, treated at the van Creveldkliniek in 2007 (n = 668) [3].

Table 21.1 Intracranial hemorrhage (ICH) according to age and hemophilia severity [10,11].

Age group	Proportion of ICH (%)	Proportion of severe hemophilia (%)
0–1 months	8.1	90.0
1–24 months	17.9	90.0
2–15 years	21.1	53.8
15–50 years	36.6	66.7
>50 years	16.3	50.0

prognosis of cardiovascular disease in nonhemophilic patients [14,15]. Furthermore, in nonhemophilic patients, traditional cardiovascular risk factors (including older age) are more prevalent in patients with chronic kidney disease.

Recommendation

In elderly hemophilia patients, especially those with hematuria in the past, renal function (creatinine) should be measured annually. In case of renal dysfunction, patients should undergo an assessment of renal function by estimating the glomerular filtration rate (GFR) and an attempt should be made to obtain a specific diagnosis. In these cases, referral to a renal specialist is warranted.

Overweight

Overweight is an increasing problem in the industrial world. A Dutch study revealed that between 1992 and 2001 the prevalence of overweight [body mass index (BMI) 25–30 kg/m^2] has increased from 27% to 35% in adult Dutch hemophilia patients [16]. However, overweight occurred significantly less frequently in hemophilia patients than in the general Dutch male population, in which the prevalence of overweight was 41% in 1992 and 50% in 2001. The prevalence of obesity (BMI >30 kg/m^2) doubled from 4% to 8%, which was not significantly decreased compared with the general population (5% in 1992 and 8% in 2001) [16].

A high BMI is associated with a significant limitation in range of motion (ROM) [17,18], and with a greater chance of developing a target joint [19]. In older patients, mobility is already reduced because of severe arthropathy caused by bleedings in the past. The lack of activity in these patients might increase the BMI, which in turn influences arthropathy again. As the BMI increases with age, the hemophilia patient has a longer life expectancy and the prevalence of obesity in the general population is foreseen, it might be expected that obesity will occur more frequently within the aging hemophilia population.

Recommendation

As increased body weight is a risk factor for development of diabetes mellitus (DM), atherosclerosis, and cardiovascular disease, and may further damage arthropathic joints because of an increase in weight bearing. It is important for hemophilia patients not to be overweighed. Therefore, regular physical activity should be advised. If functional limitations limit daily activities, a physical therapist familiar with hemophilia may be of help. In some cases referral to a dietitian may be indicated.

Diabetes mellitus

The prevalence of DM in hemophilia is not well documented. Walsh *et al.* reported a prevalence of 24% in a cohort of hemophilia patients compared with 6.1% in control males [6]. However, there have been no other studies published to confirm these findings.

Recommendation

In aging hemophilia patients, especially those who are overweight, glucose levels should be checked annually. If treatment with insulin is indicated, subcutaneous injections can be applied without bleeding complications.

Cholesterol

Mean cholesterol levels of patients with hemophilia are lower than in the general population. Patients with severe hemophilia had the lowest cholesterol levels, which would suggest an association between low cholesterol concentrations and the clotting factor deficiency or its treatment [5]. Another hypothesis is that viral infections influence both the immune system and liver function, and therefore have an effect on cholesterol levels. This is supported by the recent observation that chronic hepatitis C is associated with lower cholesterol concentrations [20].

Recommendation

In aging hemophilia patients cholesterol levels [total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) fraction] should be measured in patients who are at risk of developing cardiovascular disease. If increased, treatment is indicated. As a general rule, the total cholesterol:HDL ratio should not be higher than 8.

Osteoporosis

Wallny *et al.* studied 62 male patients with severe hemophilia, with a mean age of 41 years. Reduced bone mineral density (BMD) was found in 43.5% and osteoporosis in 25% [21]. Number and severity of arthropathic joints were associated with lower BMD in the neck of the femur. Painful hemophilic arthropathy with reduced mobility and lack of activity may lead to a reduction of bone mass. Additional risk factors were chronic HCV, low BMI, and age. These findings were confirmed in a study by Mansouritorghabeh [22].

Recommendation

Weight-bearing physical activity (sports), physical therapy, surgery to remobilize patients, and calcium and vitamin D supplementation are recommended [23].

Cardiovascular disease

Although several studies report a reduced mortality because of ischemic cardiovascular disease in hemophilia patients compared with the general age-matched male population, the number of deaths as a result of ischemic heart disease is increasing [1,2,24–26]. In the Netherlands, between 1972 and 2001, death caused by ischemic heart disease increased from 2% to 6% [2]. An American study demonstrated that the age-specific prevalence of ischemic heart disease in hemophilia patients ranged from 0.05% in those aged under 30 years to 15.2% in those aged 60 years or older [27]. Hospital discharge rates for ischemic heart disease in the USA were lower compared with age-matched males [27]. Other studies, however, found a higher prevalence of heart disease compared with a control group: 18% and 9%, respectively [6,28].

Although hypertension is more frequently seen in this patient group, hemophilia seems to protect against cardiovascular disease. A possible association between the occurrence of myocardial infarction and previous administration of clotting factor concentrates has been described [29].

The lower incidence of ischemic heart disease in patients with hemophilia has been attributed to the hypocoagulable state of these patients compared with the general population, leading to a decreased tendency to form occlusive thrombi. It remains unclear whether the deficiency of coagulation factor VIII or IX also exerts a protective effect on the development of atherosclerosis. Studies concerning intima–media thickness in hemophilia patients report conflicting results [30–32]. Using B-mode ultrasound, Srámek *et al.* found no differences in intima–media thickness of the carotid artery between patients with bleeding disorders and healthy controls. Intima–media thickness of the femoral artery was minimally reduced in patients with bleeding disorders compared with controls (adjusted difference –0.078 mm; 95% CI –0.17 to 0.018 mm). Femoral artery walls were thinnest in individuals with moderate to severe hemophilia [32]. Another study, however, showed that the mean intima–media thickness was significantly lower in 50 patients with hemophilia (38 severe, 12 moderate) compared with control subjects [31].

Recommendation

Although adequate guidelines for treatment of cardiovascular disease in hemophilia are still lacking, this should not withhold hemophilia patients from optimal cardiac care. It is a major challenge for all specialists involved.

When *cardiac intervention* is needed for coronary syndromes, percutaneous coronary intervention (PCI) with stenting is feasible in hemophilic patients. Adequate clotting factor concentrate (CFC) correction in combination with an adapted anticoagulant and antiplatelet therapeutic schedule tailored to the individual patient profile is required, taking into account severity of hemophilia, severity of cardiovascular disease, age, inhibitor status, and renal function. The balance between thrombosis and hemostasis requires a tight cooperation between hemophilia specialists and cardiologists. Both short- and long-term treatment with antiplatelet drugs should be weighed against the increased bleeding risk. In general, antiplatelet medication in patients with hemophilia is feasible, but may sometimes require an adjusted prophylaxis with CFC [33].

Stable angina pectoris in patients with mild hemophilia or those on prophylaxis with CFC can be treated with 80 mg aspirin daily. The use of aspirin should be carefully balanced against the bleeding phenotype of the patient. When bleeding frequency increases, aspirin should be stopped. Some hemophilia specialists state that clopidogrel is well tolerated in patients with mild hemophilia [3]. In our center we have used aspirin in moderate hemophilia successfully without bleeding complications.

Acute coronary syndromes in need for PCI require adequate correction with CFC. We recommend to pursue a peak level of 0.8 U/L before PCI and until 48 h after PCI. Higher levels should be avoided in order to prevent occlusive thrombi. During complete CFC correction, heparin can be administered according to standard cardiologic treatment protocols. Glycoprotein IIb/IIIa inhibitors (abciximab, tirofiban) are used in PCI with stenting and have been used in hemophilia patients [34].

We prefer to use radial artery access site for PCI instead of femoral, in order to minimize retroperitoneal or groin bleeds. If a stent is needed, we recommend a bare metal stent (BMS) instead of a drug-eluting stent, as the latter requires prolonged dual antiplatelet therapy with aspirin and clopidogrel. As dual antiplatelet therapy, we recommend an oral loading dose of 600 mg clopidogrel in hemophilia patients before PCI, followed by 75 mg clopidogrel daily for a minimum of 2 weeks after BMS, in addition to 80 mg aspirin. If possible, this should be pursued for a duration of 4 weeks. These dosages are the same as in nonhemophilic patients. However, this necessitates the use of CFC aiming at trough levels of 0.3 U/L as long as dual antiplatelet therapy is given. Regular clotting factor level measurements are required to optimize dosing. Thereafter, the patient's regular factor replacement treatment schedule can be continued.

Malignancy and surgical interventions

Except for hepatocellular carcinoma owing to chronic HCV infection, mortality rates for cancer are the same in hemophilia patients as in the general population [1,2]. With increasing age, patients with hemophilia will develop malignancies and other diseases like prostate hyperplasia, which require biopsy and in some cases surgical intervention.

Recommendation

Hemophilia is not a contraindication for medical intervention or surgery, but adequate clotting factor correction is required. Duration and dosage depend on the type of intervention or surgery and severity of hemophilia. Daily measurement of clotting factor levels helps to optimize therapy with CFC. Early mobilization is indicated, not only to prevent deep venous thrombosis, but also to prevent deterioration of preexisting arthropathy.

Prevention of deep venous thrombosis

Deep venous thrombosis is described in hemophilia patients receiving high doses of CFC during surgical interventions [35,36]. Patients undergoing surgery should be treated with thrombosis prophylaxis with low-molecular-weight heparin according to local protocols, as in patients without hemophilia. However, thrombosis prophylaxis should always start *after* complete clotting factor correction. In addition, compression stockings can be used perioperatively until the patient is fully mobilized.

Tooth extraction

Many elderly hemophilia patients lacked good dental care during their youth, owing to their clotting factor deficiencies, and have teeth which are in bad condition, often necessitating tooth extractions. This requires good coordination between dentist or oral surgeon and hematologist.

Recommendation

Clotting factor correction is dependent on the number of extractions, the shape of the gingival, and complications during extraction. For uncomplicated extractions, a single infusion with CFC, aiming at a peak level of 50%, in combination with tranexamic acid in a dosage of 1–1.5 g three times a day, will do. In patients with severe or moderate hemophilia, CFC should be repeated on the first and fifth day after extraction to prevent late bleeding. To avoid upper airway hematoma, nerve trunk infiltration and general anesthesia may be given only after complete clotting factor correction. Local application of antifibrinolytics, such as Spongostan oral®, and silk suturing may further prevent bleeding [37,38].

Sexuality

For many people sexuality is an essential part of well-being. They continue trying to find satisfactory sexual expression and intimacy. Being old in itself is no reason to give up sex. Hemophilia can be accompanied by sexual dysfunction which may include lack of sexual desire or excitement (erection) or sexual response (ejaculation) [39,40].

Pain, or fear of pain, may affect sexual desire. Hemophilic arthropathy may place limitations on sexual intercourse as well. Chronic HCV or HIV itself, or its treatment, can influence sexuality. Fear of transmission, or use of condoms, may decrease sexual desire in a patient or his partner. Hypertension, kidney disease, and heart disease may also have a negative effect.

Recommendation

Communication between healthcare professionals and patients is important to detect sexual dysfunction. As patients are

mostly too shy to bring up the subject, hemophilia care-givers should proactively do so. For counseling the PLISSIT model can be used [40]. Analgesics before sexual contact, and specific advice, including positions suitable for various joint problems, may further improve sexuality. Erection-enhancing medication may be useful. However, one should be careful with prescribing Viagra®, as this may cause bleedings.

Psychological problems

Associated with the physical aspects of arthropathy and aging, hemophilia patients become aware of, or suffer from, psychosocial problems [41]. These may be triggered by loss of work, early retirement, decline in health, or altered family dynamics. The patient's network may shrink and informal care may become a problem. Adaptations at home, increased care-giver support and eligibility of formal care-givers may help the patient to maintain functioning in the home situation. But sometimes they have to give up independent life and move to a nursing home. For patients with hemophilia, who have fought for self-determination for many years, this can be very dramatic. On the other hand, many hemophilia patients learned to overcome problems with their disease during their youth. These experiences will help them to tackle problems they meet when getting older.

Fear during hospitalization

During hospitalization patients may be confronted with unexpected emotional problems, owing to negative experiences in their youth. This may be aggravated by fear. As most patients are managers of their own disease (hemophilia), lack of control, especially during hospitalization, may cause additional stress and emotions. Furthermore, the fact that patients are used to self-infusion may be confusing for a medical staff unfamiliar with hemophilia patients. Good information and education by a hemophilia nurse is mandatory.

Quality of life

Quality of life is an important issue in aging hemophilia patients. Several studies indicate that quality of life, especially regarding physical functioning, is reduced compared with the general population, even in patients with mild hemophilia [6,42,43]. Factors that negatively influence quality of life in these patients are increasing age, severity of hemophilia, the presence of arthropathy, HCV infection, HIV infection, and unemployment [44–46]. Health-related quality of life (HRQoL) was determined in a cohort of 602 Dutch hemophilia patients [45]. Figure 21.2 shows HRQoL in the general population and in hemophilia patients aged <40 years or >40 years, with or without HCV infection. HRQoL was measured using the RAND-36 questionnaire, assessing eight domains of HRQoL: Physical functioning, social functioning, role physical (difficulties in daily activities because of physical health problems), role emotional (difficulties in daily activities because of emotional problems), mental health, vitality, bodily pain, and general health. Higher scores indicate better quality of life. In hemophilia patients in general, HRQoL was significantly lower for patients aged ≥40 years than for patients aged <40 years in all domains (*P*-value of the Mann–Whitney *U*-test ≤0.001). In patients with a current HCV infection, the only nonsignificant difference between the two age groups occurred in the mental health domain ($P = 0.07$). All other domains showed significantly worse HRQoL in HC-infected patients aged ≥40 years than in patients aged <40 years (adjusted from Posthouwer *et al.* [45]).

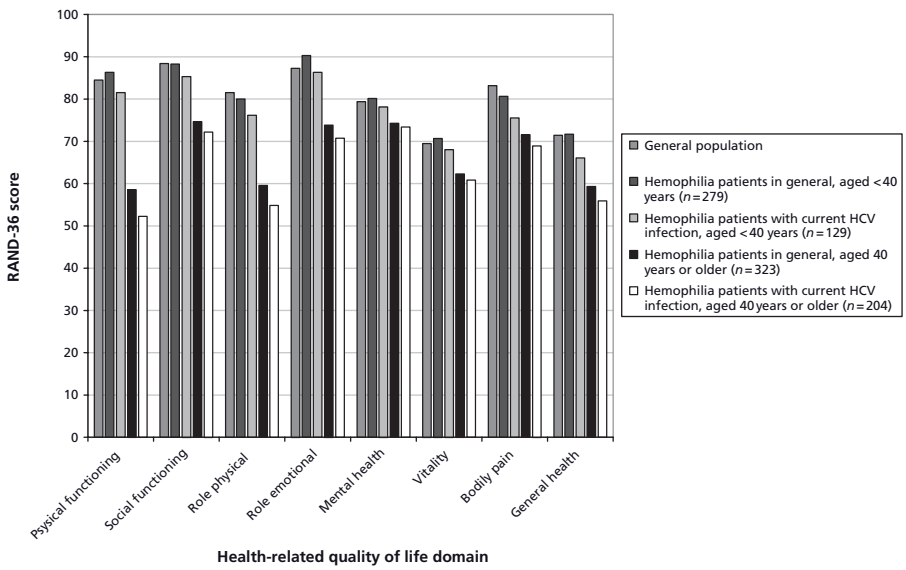

Figure 21.2 Health-related quality of life in hemophilia patients in general, and in a subgroup of hemophilia patients with a current hepatitis C virus infection, divided in different age groups, compared with the general population. Higher RAND-36 scores indicate better quality of life. Adjusted from [45].

Table 21.2 Guidelines for the use of analgesics for hemophilic arthropathy.

Paracetamol (500–1000 mg, maximum six times a day) is the initial medication of choice. If not effective:
paracetamol and a muscle relaxant (diazepam 5 mg, one to three times a day), or
paracetamol and codeine (10–20 mg, maximum six times a day)
Tramadol is indicated for very severe pain (50–100 mg, three or four times a day)
Morphine: use a slow-release product, starting with 20 mg two times a day, with an escape of a rapid-release product 10 mg four times a day. Increase the slow-release product if the rapid release product is used more than four times a day

Pain

Pain has a negative effect on quality of life. Wallny *et al.* studied pain in a group of 91 adult hemophilia patients with a mean age of 43 years. On average, they had four joints with major pain: Ankle (45%), knee (39%), elbow (7%), and hip (6%) [47]. Fourteen percent of patients complained of distressing pain in the spine. Fifty percent of patients had pain throughout the day, when no treatment was given. These findings were confirmed by van Genderen *et al.*, who found that 36% of patients who indicated to have pain used analgesics [48].

Recommendation

Since pain has an impact not only on quality of life, but also on daily functioning, it has to be addressed adequately [44,48]. Treatment consists of pain medication, distal traction, transcutaneous electrical nerve stimulation (TENS), and hot packs.

Adequate pain medication can be prescribed according to the guideline in Table 21.2. Since codeine and morphine often lead to constipation, prescription of a laxating drug is mandatory. Morphine may cause nausea, which improves with time and can be treated with rectal metoclopramide.

Diclophenac and other nonsteroidal anti-inflammatory drugs (NSAIDs) are theoretically contraindicated and should, in general, be avoided, because they may affect platelet function and may increase bleeding tendency. However, some patients with chronic arthropathic pain may benefit from ibuprofen, without bleeding complications [49]. Although others have reported increased bruising and gastrointestinal bleeding [50,51], Rattray *et al.* reported a positive effect of cyclooxygenase 2 (COX-2) inhibitors on chronic pain in a small group of patients with hemophilia [52]. When given in commonly used doses, COX-2 inhibitors do not increase the risk of cardiovascular disease [53].

Balance dysfunctions and risk of falls

Falls are associated with increased morbidity, mortality, and referral to nursing homes. So far, there is little literature on the problem of falling in patients with hemophilia. A study on this subject was initiated by Street *et al.* [41]. The results are still pending. According to Rao *et al.*, risk factors for falls include muscle weakness, a history of falls, arthritis (especially of the knee), and impairment in gait and activities in daily living [54]. In general, patients with hemophilic arthropathy have several of these risk factors.

Recommendation

The most effective preventive strategies are multifactorial interventions targeting identified risk factors, balance training, and exercises for muscle strength [41,54].

Conclusion

Comorbidity is quite common in the latter phase of human life, and hemophilia patients are no exception. It may lead to an increase in functional limitations, psychosocial complaints and symptoms, social and societal problems, and a decrease in quality of life. Besides adequate treatment of hemophilic arthropathy and HCV and HIV infection, it is also important to look for ways to prevent or reduce comorbidity and improve quality of life. Hemophilia care-givers should play a role in this and during annual check-ups pay attention not only to hematologic aspects of hemophilia, but also to age-related comorbidity and psychosocial problems.

Comorbidity in hemophilia patients may lead to complex treatment. Lack of coordination between various healthcare workers may result in slow and ponderous healthcare delivery services, uncontrolled polypharmacy, and further loss of patients' well-being and quality of life. Mortality rates among hemophilia patients are higher in patients not treated in hemophilia centers [28]. Hemophilia centers should have an important part in coordinating care for these patients, not only when they are admitted to hospital, but also by supporting care and needs in the domestic environment. This may help to improve quality of life and to maintain independence.

References

1 Darby SC, Kan SW, Spooner RJ, *et al.* Mortality rates, life expectancy, and causes of death in people with hemophilia A or B in the United Kingdom who were not infected with HIV. *Blood* 2007; **110**: 815–25.
2 Plug I, Van Der Bom JG, Peters M, *et al.* Mortality and causes of death in patients with hemophilia, 1992–2001: A prospective cohort study. *J Thromb Haemost* 2006; **4**: 510–516.
3 Mauser-Bunschoten EP, De Knecht-van Eekelen A, Smit C. *Aging with haemophilia—Medical and Psychosocial Impact*. Utrecht: Van Creveldkliniek-Haematology, 2007.
4 Mauser-Bunschoten EP, Fransen van de Putte DE, Schutgens REG. Co-morbidity in the ageing haemophilia patient; the down side of increased life expectancy. *Haemophilia* 2009; **15**: 853–63.

5 Rosendaal FR, Briet E, Stibbe J, van Herpen G, Leuven JA, Hofman A, Vandenbroucke JP. Haemophilia protects against ischaemic heart disease: A study of risk factors. *Br J Haematol* 1990; **75**: 525–30.

6 Walsh M, Macgregor D, Stuckless S, Barrett B, Kawaja M, Scully MF. Health-related quality of life in a cohort of adult patients with mild hemophilia A. *J Thromb Haemost* 2008; **6**: 755–61.

7 White GC, Blatt PM, McMillan CW, Webster WP, Lesesne HR, Roberts HR. Medical complications of hemophilia. *South Med J* 1980; **73**: 155–60.

8 Prentice CR, Lindsay RM, Barr RD, Forbes CD, Kennedy AC, McNicol GP, Douglas AS. Renal complications in haemophilia and Christmas disease. *Q J Med* 1971; **40**: 47–61.

9 Rosendaal FR, Varekamp I, Smit C, *et al*. Mortality and causes of death in Dutch haemophiliacs, 1973–86. *Br J Haematol* 1989; **71**: 71–76.

10 Stieltjes N, Calvez T, Demiguel V, *et al*. Intracranial haemorrhages in French haemophilia patients (1991–2001): Clinical presentation, management and prognosis factors for death. *Haemophilia* 2005; **11**: 452–8.

11 Ljung RC. Intracranial haemorrhage in haemophilia A and B. *Br J Haematol* 2008; **140**: 378–84.

12 Beck P, Evans KT. Renal abnormalities in patients with haemophilia and Christmas disease. *Clin Radiol* 1972; **23**: 349–54.

13 Kulkarni R, Soucie JM, Evatt B. Renal disease among males with haemophilia. *Haemophilia* 2003; **9**: 703–10.

14 Sarnak MJ, Levey AS, Schoolwerth AC, *et al*. Kidney disease as a risk factor for development of cardiovascular disease: A statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 2003; **108**: 2154–69.

15 Muntner P, He J, Hamm L, Loria C, Whelton PK. Renal insufficiency and subsequent death resulting from cardiovascular disease in the United States. *J Am Soc Nephrol* 2002; **13**: 745–53.

16 Hofstede FG, Fijnvandraat K, Plug I, Kamphuisen PW, Rosendaal FR, Peters M. Obesity: A new disaster for haemophilic patients? A nationwide survey. *Haemophilia* 2008; **14**: 1035–8.

17 Scully MF, Macgregor D, Walsh M, Barrett B, Kawaja M, Sheppard C. The clinical impact of mild hemophilia A, report of a cohort study. *Blood (ASH Annual Meeting Abstracts)* 2005; **106**: abstract 3210.

18 Soucie JM, Cianfrini C, Janco RL, *et al*. Joint range-of-motion limitations among young males with hemophilia: Prevalence and risk factors. *Blood* 2004; **103**: 2467–73.

19 Carpenter SL, Chrisco M, Johnson E. The effect of overweight and obesity on joint damage in patients with moderate or severe hemophilia. *Blood* 2006; **108**: 4064.

20 Wisniewska-Ligier M, Wozniakowska-Gesicka T, Kups J, Sulat-Syncerek D. Lipid metabolism in children with chronic hepatitis C, A preliminary report. *Hepatogastroenterology* 2006; **53**: 887–91.

21 Wallny TA, Scholz DT, Oldenburg J, *et al*. Osteoporosis in haemophilia—An underestimated comorbidity? *Haemophilia* 2007; **13**: 79–84.

22 Mansouritorghabeh H, Rezaieyazdi Z, Badiei Z. Are individuals with severe haemophilia A prone to reduced bone density? *Rheumatol Int* 2008; **28**: 1079–83.

23 Kovacs CS. Hemophilia, low bone mass, and osteopenia/osteoporosis. *Transfus Apher Sci* 2008; **38**: 33–40.

24 Aronson DL. Cause of death in hemophilia A patients in the United States from 1968 to 1979. *Am J Hematol* 1988; **27**: 7–12.

25 Koumbarelis E, Rosendaal FR, Gialeraki A, *et al*. Epidemiology of haemophilia in Greece: An overview. *Thromb Haemost* 1994; **72**: 808–13.

26 Larsson SA, Wiechel B. Deaths in Swedish hemophiliacs, 1957–1980. *Acta Med Scand* 1983; **214**: 199–206.

27 Kulkarni R, Soucie JM, Evatt BL. Prevalence and risk factors for heart disease among males with hemophilia. *Am J Hematol* 2005; **79**: 36–42.

28 Soucie JM, Nuss R, Evatt B, *et al*. Mortality among males with hemophilia: Relations with source of medical care. The Hemophilia Surveillance System Project Investigators. *Blood* 2000; **96**: 437–42.

29 Girolami A, Ruzzon E, Fabris F, Varvarikis C, Sartori R, Girolami B. Myocardial infarction and other arterial occlusions in hemophilia a patients. A cardiological evaluation of all 42 cases reported in the literature. *Acta Haematol* 2006; **116**: 120–5.

30 Bilora F, Boccioletti V, Zanon E, Petrobelli F, Girolami A. Hemophilia A, von Willebrand disease, and atherosclerosis of abdominal aorta and leg arteries: Factor VIII and von Willebrand factor defects appear to protect abdominal aorta and leg arteries from atherosclerosis. *Clin Appl Thromb Hemost* 2001; **7**: 311–13.

31 Bilora F, Zanon E, Petrobelli F, Cavraro M, Prandoni P, Pagnan A, Girolami A. Does hemophilia protect against atherosclerosis? A case–control study. *Clin Appl Thromb Hemost* 2006; **12**: 193–8.

32 Sramek A, Reiber JH, Gerrits WB, Rosendaal FR. Decreased coagulability has no clinically relevant effect on atherogenesis: Observations in individuals with a hereditary bleeding tendency. *Circulation* 2001; **104**: 762–7.

33 Tuinenburg A, Mauser-Bunschoten EP, Verhaar MC, Biesma DH, Schutgens REG. Cardiovascular disease in patients with haemophilia. Accepted for publication in *J Thromb Haemost* 2009; **7**: 247–54.

34 Bovenzi F, De Luca L, Signore N, Fusco F, De Luca I. Abciximab for the treatment of an acute thrombotic coronary occlusion during stent implantation in a patient with severe hemophilia B. *Ital Heart J* 2003; **4**: 728–30.

35 Lowe GD. Factor IX and thrombosis. *Br J Haematol* 2001; **115**: 507–13.

36 Ritchie B, Woodman RC, Poon MC. Deep venous thrombosis in hemophilia A. *Am J Med* 1992; **93**: 699–700.

37 Jover-Cervero A, Poveda Roda R, Bagan JV, Jimenez Soriano Y. Dental treatment of patients with coagulation factor alterations: An update. *Med Oral Patol Oral Cir Bucal* 2007; 12: E380–E387.

38 Piot B, Sigaud-Fiks M, Huet P, Fressinaud E, Trossaert M, Mercier J. Management of dental extractions in patients with bleeding disorders. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **93**: 247–50.

39 Gianotten WL, Heijnen L. Haemophilia, aging and sexuality. *Haemophilia* 2009; **15**: 55–62.

40 Parish KL. Sexuality and haemophilia: Connections across the life-span. *Haemophilia* 2002; **8**: 353–9.

41 Street A, Hill K, Sussex B, Warner M, Scully MF. Haemophilia and ageing. *Haemophilia* 2006; **12**(Suppl. 3): 8–12.

42 Szende A, Schramm W, Flood E, Larson P, Gorina E, Rentz AM, Snyder L. Health-related quality of life assessment in adult haemophilia patients: A systematic review and evaluation of instruments. *Haemophilia* 2003; **9**: 678–87.

43 Talaulikar D, Shadbolt B, McDonald A, Pidcock M. Health-related quality of life in chronic coagulation disorders. *Haemophilia* 2006; **12**: 633–42.

44 Fischer K, Van Der Bom JG, Mauser-Bunschoten EP, Roosendaal G, van den Berg HM. Effects of haemophilic arthropathy on health-related quality of life and socio-economic parameters. *Haemophilia* 2005; **11**: 43–48.
45 Posthouwer D, Plug I, Van Der Bom JG, Fischer K, Rosendaal FR, Mauser-Bunschoten EP. Hepatitis C and health-related quality of life among patients with hemophilia. *Haematologica* 2005; **90**: 846–50.
46 Trippoli S, Vaiani M, Linari S, Longo G, Morfini M, Messori A. Multivariate analysis of factors influencing quality of life and utility in patients with haemophilia. *Haematologica* 2001; **86**: 722–8.
47 Wallny T, Hess L, Seuser A, Zander D, Brackmann HH, Kraft CN. Pain status of patients with severe haemophilic arthropathy. *Haemophilia* 2001; **7**: 453–8.
48 van Genderen FR, Fischer K, Heijnen L, de Kleijn P, van den Berg HM, Helders PJ, van Meeteren NL. Pain and functional limitations in patients with severe haemophilia. *Haemophilia* 2006; **12**: 147–53.
49 Steven MM, Small M, Pinkerton L, Madhok R, Sturrock RD, Forbes CD. Non-steroidal anti-inflammatory drugs in haemophilic arthritis. A clinical and laboratory study. *Haemostasis* 1985; **15**: 204–9.
50 Cagnoni PJ, Aledort L. Gastrointestinal bleeding in hemophilia as a complication of the use of over the counter non-steroidal anti-inflammatory drugs. *Am J Hematol* 1994; **47**: 336–7.
51 Daly HM, Scott GL. Extensive ecchymoses due to ibuprofen therapy in the management of haemophiliac arthropathy. *Clin Lab Haematol* 1984; **6**: 85–7.
52 Rattray B, Nugent DJ, Young G. Celecoxib in the treatment of haemophilic synovitis, target joints, and pain in adults and children with haemophilia. *Haemophilia* 2006; **12**: 514–17.
53 McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: A systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. *JAMA* 2006; **296**: 1633–44.
54 Rao SS. Prevention of falls in older patients. *Am Fam Physician* 2005; **72**: 81–88.

22 Products used to treat hemophilia: recombinant products

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After the human factor VIII (FVIII) gene was cloned and expressed in cell culture by two independent biotechnology groups in 1984, two pharmaceutical companies developed large-scale production methods for full-length, recombinant FVIII (rFVIII) preparations (Kogenate® by Miles-Cutter/Bayer Corporation and Recombinate® by Baxter Healthcare). These were licensed in the early 1990s [1,2].

Interest in developing genetically engineered recombinant clotting factors was driven by the desire for "safer" therapeutic products following the tragic epidemics of blood-borne transmitted diseases in the 1970s and 1980s. The main advantages of these recombinant products are viral safety, independence from plasma supply, and very small volume.

Following intensive preclinical evaluation, clinical trials were undertaken worldwide, which demonstrated that rFVIII is comparable to plasma-derived (pd) FVIII not only in the characteristics of FVIII itself but also in the safety and efficacy of treatment for hemorrhagic episodes. Subsequently, no significant adverse events have been reported.

The first-generation rFVIII proteins were stabilized with bovine or human serum albumin (HSA) either in preparation or in final formulation. Although no viral transmissions have been documented and confirmed with albumin after more than 50 years of clinical use, manufacturers nevertheless worked toward developing products contains minimum amounts of human- or animal-derived components.

Thus, second-generation therapies have been produced in which the HSA in the final vial is replaced by nonprotein stabilizers. The second-generation types were ReFacto®, produced by Genetics Institute/Wyeth, and Kogenate FS®/Kogenate Bayer®, produced by Bayer Corporation.

Third-generation products lack added bovine and/or human protein in either the cell culture procedure or the final vial. The third-generation types are BeneFIX® (rFIX, produced by Genetics Institute/Wyeth), Advate® (rFVIII, by Baxter Bioscience) and Xyntha® (rFVIII, by Wyeth) (Table 22.1).

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Recombinant factor VIII: Kogenate (Helixate), Kogenate FS (Kogenate Bayer, Helixate FS/NexGen), and Kogenate FS BIO-SET

An established baby hamster kidney (BHK) cell line was transfected with human FVIII cDNA and secreted full-length rFVIII into the culture medium without the addition of von Willebrand factor (VWF) [2]. The secreted rFVIII was then subjected to multiple purification steps, including ion-exchange, size exclusion, and immunoaffinity chromatography using a murine monoclonal anti-FVIII antibody. Thus, Kogenate contained trace amounts of hamster protein (51 ng/1000 IU of FVIII:C) and murine immunoglobulin G (IgG) (1–27 ng/1000 IU of FVIII:C) from the manufacturing process, as well as HSA (100 mg/1000 IU of FVIII:C) as stabilizer [3]. The purification steps for Kogenate had the capacity to remove and inactivate viruses even though the cultures were believed to be virus free. The whole process, which included chromatography steps and heat treatment, was validated for a 12-log reduction of relevant model viruses.

Kogenate FS/Kogenate Bayer (Helixate FS/NexGen®, distributed by CSL Behring through a license agreement) are produced as second-generation concentrates using the same production cell line and cell culture process as the first-generation Kogenate. A solvent/detergent (S/D) stage using tri-N-butyl phosphate and Triton X-100 has been included early in the preparation. Unlike the original Kogenate, the second-generation products are stabilized in the final formulation which sucrose prior to lyophilization. In validated viral spiking studies, the manufacturing process of Kogenate FS has been shown to have the potential to reduce model enveloped viruses by >16.4 logs and to remove the prions responsible for the transmissible spongiform encephalopathies (TSEs) [4]. The BIO-SET reconstitution system for Kogenate FS was first launched in 2005.

Clinical trials in previously treated patients

Stage I safety and pharmacokinetic studies and stage II safety and efficacy studies in 56 previously treated patients (PTPs)

Table 22.1 Summary of recombinant FVIII and FIX products commercially available (2008).

Product	Manufacturer/ distributor	Cell line	Gene	Protein in culture medium	Murine MAbs	Stabilizer in final vial	Viral inactivation/ removal	Generation
Kogenate FS® Kogenate Bayer® Kogenate FS BIO-SET®	Bayer Corp.	BHK	FVIII	HSA	Yes	Sucrose	SD	2
Helixate FS® Helixate NexGen®	CSL Behring							
Recombinate®	Baxter	CHO	FVIII, VWF	BSA, Insulin, Aprotinin	Yes	HSA	Heat	1
Bioclate®	CSL Behring							
Advate®	Baxter	CHO	FVIII, VWF	No	Yes	Sugar, etc.	SD	3
ReFacto®	(Wyeth)	CHO	BDDFVIII	HSA	Yes	Sucrose Histidine Polysorbate 80	SD	2
Xyntha®	Wyeth	CHO	BDDFVIII	No	No	Sucrose Histidine Polysorbate 80	NF	3
BeneFIX®	Wyeth	CHO	FIX	No	No	Sucrose Histidine Glycine Polysorbate 80 Vitamin K	NF	3

BDD, B domain deleted; BHK, baby hamster kidney; BSA, bovine serum albumin; CHO, Chinese hamster ovary; HSA, human serum albumin; MAbs, monoclonal antibodies; NF, nanofiltration; SD, solvent/detergent.

showed that Kogenate was safe and clinically effective for the treatment and prevention of hemorrhage in hemophilia A patients and that its behavior *in vitro* was similar to that of pdFVIII concentrates [2].

The first clinical trials of a sucrose-formulated full-length rFVIII-FS (Kogenate FS) were conducted in 35 PTPs with severe hemophilia A (FVIII:C < 2 IU/dL) in North America and Europe. RFVIII-FS displayed a pharmacokinetic profile similar to that of Kogenate. Safety and efficacy during home treatment were evaluated in 71 patients. Of 2585 bleeding episodes, 93.5% were treated with one or two infusions and 80.5% of responses were rated as excellent or good [5]. Subsequently, several studies have been followed and demonstrated the similar efficacy and safety of the preparation during surgical procedures and normal clinical use for long-term treatment of PTPs [6,7]. Low incidence (0–0.9%) of *de novo* inhibitor development was observed [5,7].

Clinical trials in previously untreated patients

In January 1989, a study of Kogenate in 101 previously untreated patients (PUPs) was initiated. Follow-up was evaluated every 3 months. At the end of December 1996, the cohort had been monitored for up to 8 years (median 4.8 years) since first exposure to rFVIII. Although the response to treatment was judged excellent and the product was well tolerated, the development of FVIII inhibitors in 21 (21/101, 20.8%; 19 of 64 severe and 2 of 16 moderate cases) of PUPs caused concern. The cumulative probability of inhibitor development was 24.8% by day 451 after first infusion with Kogenate and 36.1% after 18 exposure days [8] (Figure 22.1).

Twelve of the 21 children developing inhibitors had high titers (>10 BU/mL) and nine had low titers. The median exposure was 9 days (range 3–41 days). Inhibitors in seven of the children with low-titer and one with high-titer antibodies were transient, disappearing despite continued episodic treatment with Kogenate. Five of eight with high-titer inhibitors were placed on immune tolerance induction (ITI) protocols with Kogenate and had an excellent response [9]. Kogenate was licensed for use in the USA in early 1993.

A prospective, international clinical trial of rFVIII-FS was conducted in 31 PUPs and minimally treated patients (MTPs) with severe hemophilia A in home therapy and surgery [10]. No unexpected drug-related adverse events were observed. The incidence of inhibitor formation (4/31, 12.9%) was consistent with previous experience with other rFVIII or pdFVIII preparations. Another large study was conducted in 37 PUPs and 24 MTPs in North America and Europe [11]. Fifteen percent (9/60) presented with an inhibitor titer ≥0.6 BU/mL during the study.

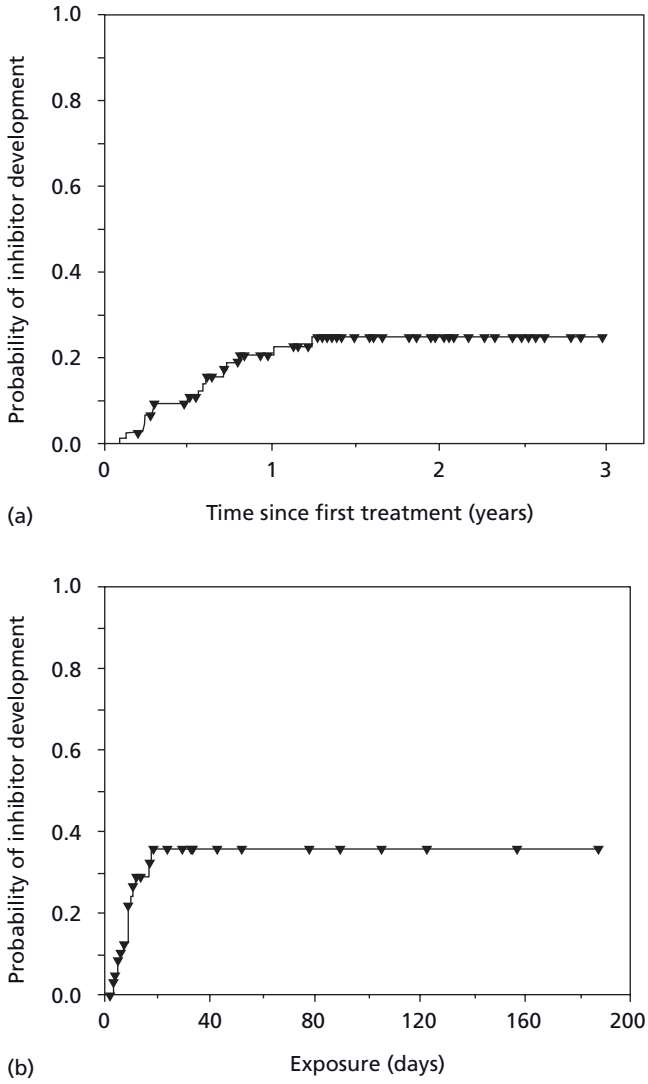


Figure 22.1 Cumulative probability of development of FVIII inhibitor in 77 previously untreated patients with severe or moderate hemophilia A from the time of initial treatment with Kogenate® (a) and according to the number of days of exposure to FVIII treatment (b). Each triangle denotes a patient without inhibitor formation. From Lusher *et al.* [8] with permission.

Recombinant factor VIII: Recombinate (Bioclate) and Advate

The synthesis of Recombinate differs from that of Kogenate in several aspects. Recombinate is manufactured in Chinese hamster ovary (CHO) cells that are cotransfected with cDNAs for both human FVIII and VWF. Cotransfection is found necessary to enhance the yield and to stabilize rFVIII [12]. The rFVIII in the conditioned medium contains added bovine proteins and is then purified by a combination of immunoaffinity and ion-exchange chromatography. VWF is removed by the same method as that used with Hemophil M (Baxter Healthcare). HSA is added to the final product as stabilizer. Recombinate is heat-treated to 40°C for 8 h in the presence of polysorbate 80 and imidazole. The production process has been validated to inactivate or exclude seven logs of relevant model viruses.

An advanced category, full-length rFVIII, plasma/albumin-free (rFVIII-PFM) preparation (named Advate) has been developed by Baxter Bioscience, without the need for additional animal- or human-derived materials in cell culture, purification, or final formulation [13]. The production of this third-generation concentrate is based largely on the original Recombinate purification process.

Clinical trials in previously treated patients

Recombinate was first infused into two PTPs with severe hemophilia A in 1987. Both patients tolerated the product well and both received the rFVIII at home for a year with clinical success and no adverse reactions [1].

Subsequently, a prospective, open-label clinical study was conducted. Pharmacokinetic studies demonstrated consistent *in vivo* recovery and biologic half-life over time (14.7 h) similar to those of pdFVIII (Hemofil M).

In Recombinate phase II studies in 55 PTPs, excellent clinical responses were noted and no FVIII inhibitors developed during >18 months' observation. The response to home treatment was judged as excellent or good in 3195 (91.2%) of 3481 bleeding episodes evaluated. Hemostasis was excellent in all 24 cases with surgical procedures [14].

Pivotal Advate phase II/III studies and its continuation studies have suggested that Advate is bioequivalent to Recombinate based on pharmacokinetic parameters and immunogenicity. They have also showed excellent to good hemostatic efficacy of bleeding episodes and safety [15]. Hemostatic evaluation of Advate in 65 surgical procedures was performed in 58 PTPs. Bolus infusion was used exclusively in 47 procedures and continuous infusion in 18. Hemostatic efficacy was assessed as excellent or good for 100% of intraoperative as well as postoperative rating [16].

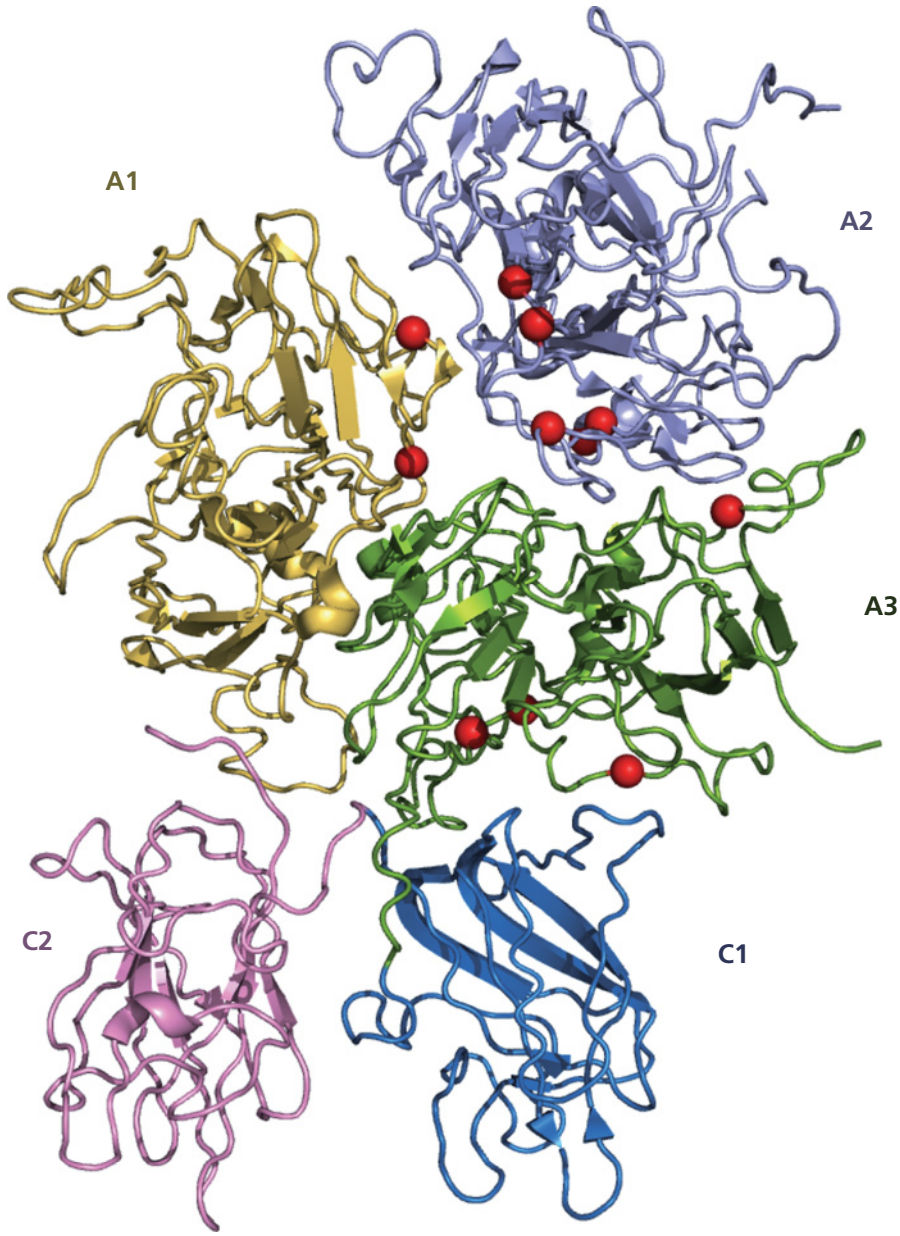
Clinical trials in previously untreated patients

A Recombinate PUPs study commenced in 1990. Seventy-nine patients with severe hemophilia A (FVIII:C < 2 U/dL) were enrolled. Hemostatic response was excellent with 92% of bleeding episodes responding to one or two infusions [17].

Inhibitor antibodies occurred in 22 (30.6%) out of the assessable 72 PUPs, nine with peak titer ≥5 BU/mL and 13 with titer <5 BU/mL. Survival analysis showed that the probability of remaining inhibitor-free in this group of patients was 88.4% after 8 exposure days, 73.6% after 10 exposure days, and 61.6% after 25 exposure days (Figure 22.2). The inhibitor disappeared in 12 patients (11 with low titers) and postinfusion recovery values were normalized.

Recombinate was licensed for use in USA in December 1992. Bioclate was distributed by Aventis Behring through a

Plate 3.3 Ribbons representation of the crystal structure of B-domain-deleted factor VIII. The positions of mutations associated with classical assay discrepancy are shown by red spheres. These cluster in two groups: at the interface between the A2 domain and the A1 and A3 domains and at the interface between the A3 and C1 domains. PDB ID: 2R7E.



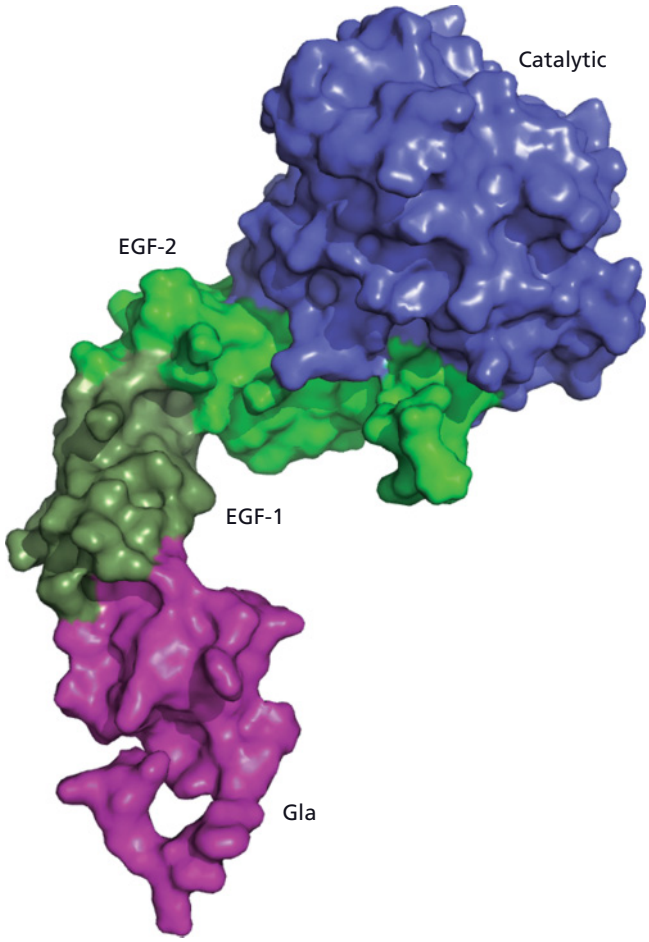


Plate 13.2 Crystal structure of porcine activated factor IX showing the domains in various shades of grey. PDB ID: 1PFX.

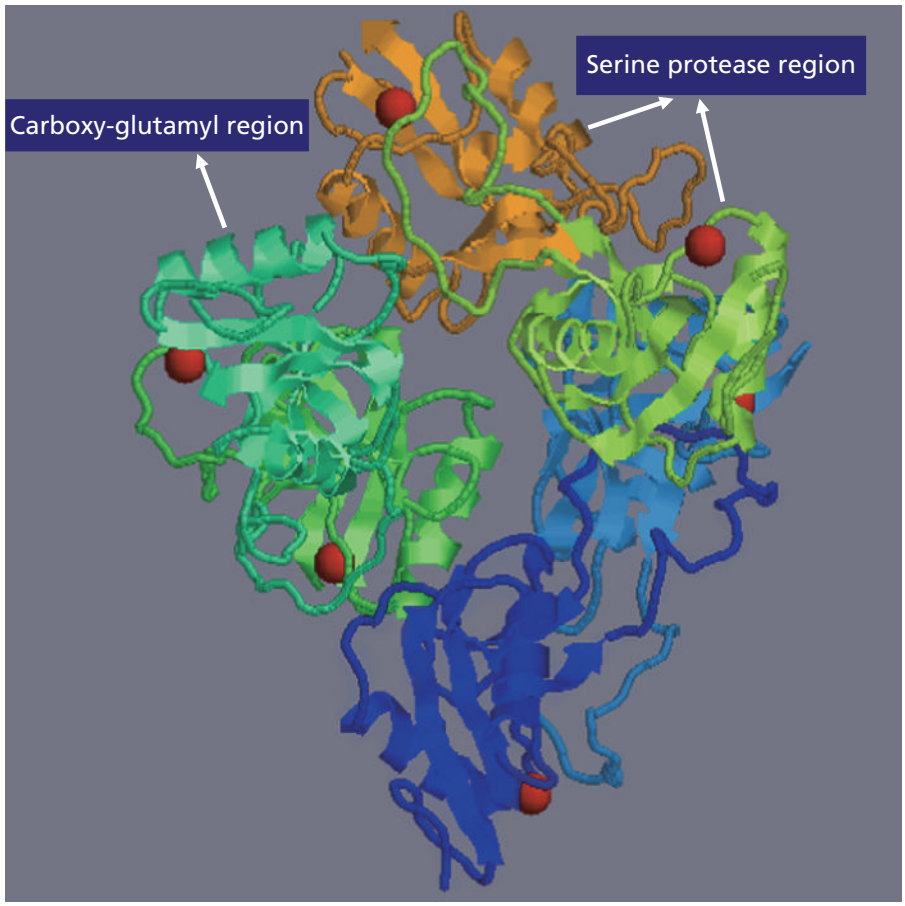
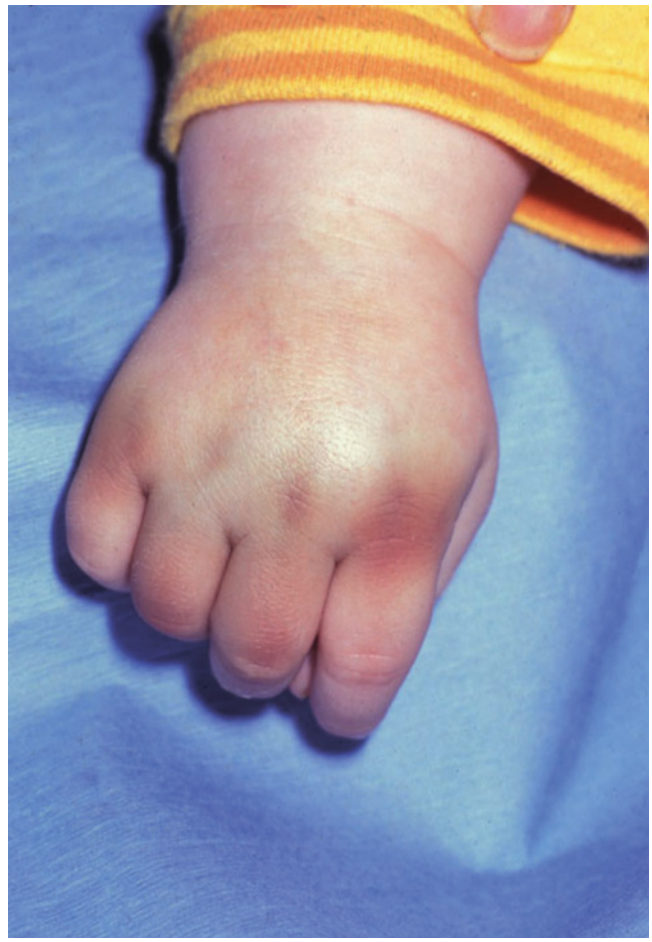


Plate 15.1 Factor IX structure illustrating antibody-binding epitopes.

Plate 17.3 Bruising secondary to hand being held firmly for venipuncture in a neonate with severe hemophilia.



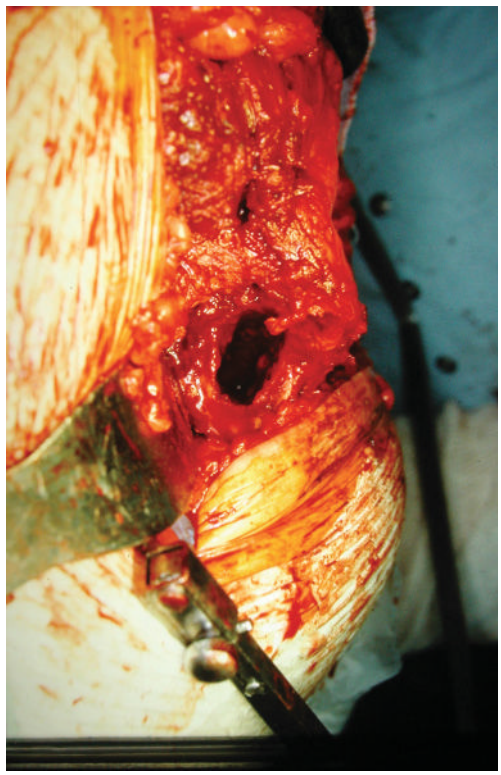


Plate 29.1 The intraoperative view demonstrates clearly a large hole in the ileum where the pseudotumor penetrated the cortex.

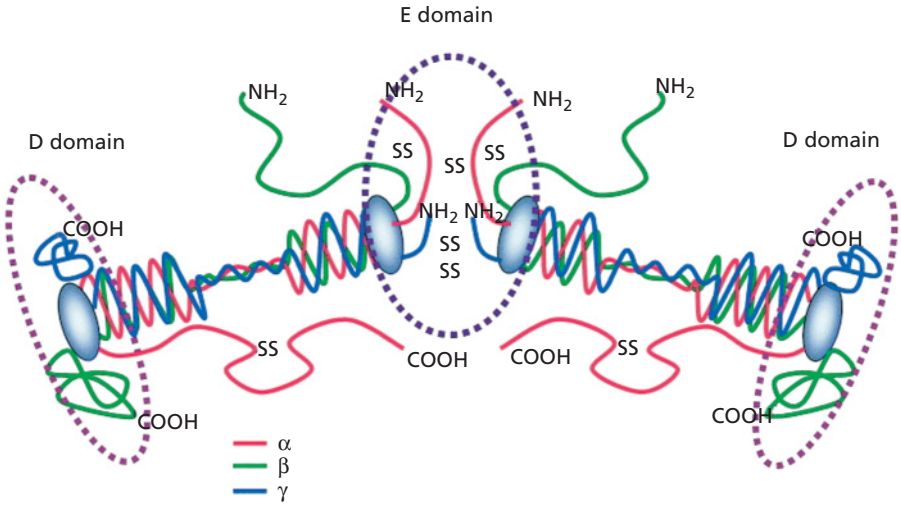


Plate 55.1 A schematic representation of the fibrinogen molecule. NH_2 and COOH denote the amino and carboxy-termini of the α-chain (red), β-chain (blue), and γ-chain (green), respectively. The free carboxy-terminal of the α-chain is referred to as Cα. The variant g′ chain extends from the carboxy-terminus of the g chain (not shown). SS identifies disulfide bonds. The E region is located in the central region of the molecule and is roughly composed of amino acids 1–49 of the α-chain, 1–80 of the β-chain, and 1–23 of the γ chain. The E region is flanked by two D regions roughly composed of amino acids 111–197 of the α-chain, 134–461 of the β-chain, and 88–406 of the γ-chain. From: Roberts HR, Stinchcombe TE, Gabriel DA. The dysfibrinogenaemias. *Br J Haematol* 2001; **114**: 249–57.

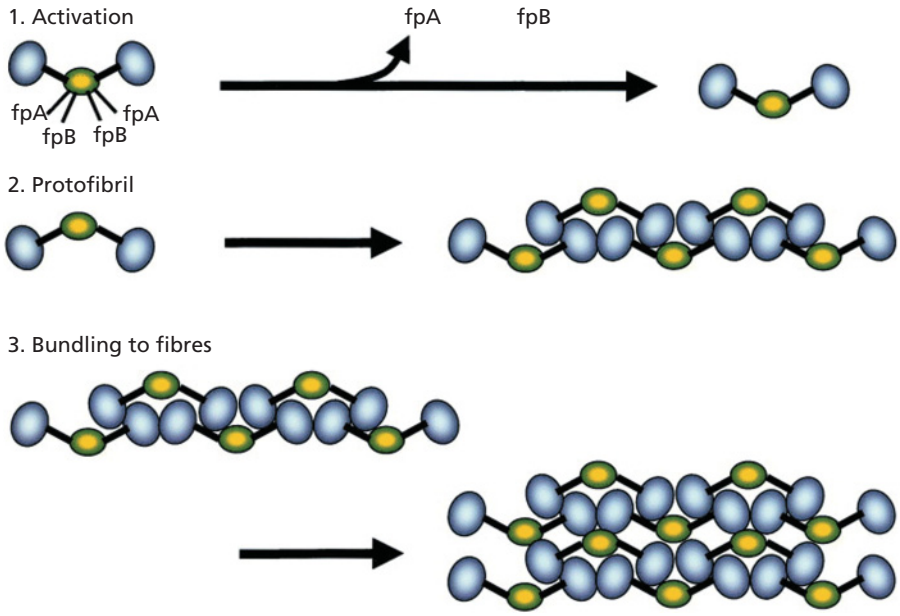


Plate 55.2 A schematic representation of the conversion of fibrinogen to a fibrin clot by thrombin. Thrombin cleavage of fibrinopeptide A (amino acids 1–16 of the Aα chain) followed by cleavage of fibrinopeptide B (amino acids 1–14 of the Bβ chain) leads to formation of fibrin monomers. Fibrin monomers then self-assemble in a half-staggered linear overlap to form protofibrils. Protofibril formation is governed by the interaction of the thrombin-exposed "a" site on the E region with "A" site on the D region. The protofibrils are then bundled into fibrin fibers through interaction of the "b" site on the E region with the "B" site located on the D region. From: Roberts HR, Stinchcombe TE, Gabriel DA. The dysfibrinogenaemias. *Br J Haematol* 2001; **114**:249–57

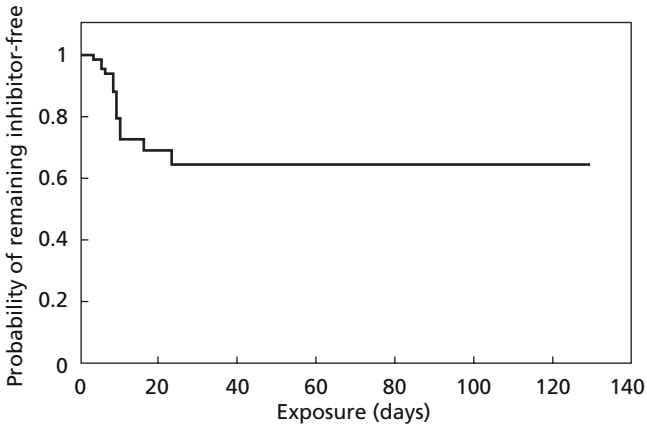


Figure 22.2 Probability of remaining inhibitor-free as a function of Recombinate® exposure days for 72 assessable study subjects. From Bray *et al.* [17] with permission.

license agreement. The third-generation rFVIII-PFM preparation, Advate, was licensed for use in the USA in 2003.

Recombinant factor VIII: ReFacto and Xyntha

The heavily glycosylated B domain of FVIII seems to be unnecessary for hemostatic activity of the FVIII molecule [18]. B-domain-deleted rFVIII (BDDrFVIII, rFVIIISQ) is more readily secreted by CHO cells and is much more stable because of reduced susceptibility to proteolysis. Thus, HSA is not needed as stabilizer in the final product, although it is utilized in the cell culture medium.

rFVIIISQ, trade name ReFacto (Wyeth), is essentially identical to pdFVIII in terms of functional properties and VWF-binding kinetics [19]. ReFacto was licensed in Europe in 1998 and in the USA in 2000. The production process includes virus inactivation using solvent/detergent (S/D) and immunoaffinity chromatography. Very recently, Xyntha (Wyeth) has been developed through modification of plasma/albumin-free manufacturing process for ReFacto and was licensed in the USA, Canada, and New Zealand. Viral safety is also enhanced by the introduction of a nanofiltration step and purification step using a chemically synthesized peptide affinity ligand instead of the usual murine monoclonal antibody [20].

One of the characteristics of rFVIIISQ (ReFacto and Xyntha) appears to be that the usual one-stage clotting assays for FVIII:C based on the activated partial thromboplastin time (aPTT) give much lower than expected recovery values in recipients (on average, 50% less). In contrast, chromogenic substrate assays give expected recovery values. It has therefore been recommended that one-stage assays should be performed in recipients of rFVIIISQ using a vial of the therapeutic material as reference standard, thus essentially adopting a "like versus like" assay principle [21]. Both rFVIIISQ employ a novel delivery system, in which various potencies are prepackaged in self-contained syringes.

Clinical trials in previously treated patients

Phase I and phase II prelicense clinical studies of rFVIIISQ (ReFacto) were conducted in 1993 and 1994 in patients with severe hemophilia A. These demonstrated that ReFacto had a pharmacokinetic profile equivalent to full-length, monoclonal antibody-purified pdFVIII (Octonativ-M, Pharmacia) and was similar in hemostatic efficacy and safety [22].

By January 1998, 113 PTPs (median age 26 years, range 8–73) had been followed in Europe and the USA [22]. In this study, 73% of episodes were resolved with one infusion, and 94% were resolved with one to three infusions. Thus, it was concluded that ReFacto was safe and efficacious for PTPs with hemophilia A for on-demand and/or prophylactic treatment. One PTP (0.9%, 1/113) developed an inhibitor with a peak titer of 13 BU/mL after 113 exposure days and 3 years into the study. Hemostatic efficacy in 37 major and 34 minor surgical procedures using ReFacto was rated excellent in all cases, and blood loss during surgery was similar to that observed in no hemophiliacs undergoing the same types of procedures [22].

A study evaluating the pharmacokinetic characteristics of Xyntha compared with those of ReFacto in a single-dose, randomized, double-blind, and two-period crossover study indicated that Xyntha was equivalent to ReFacto [23]. Subsequently, an open-label study was conducted to demonstrate the safety and efficacy of Xyntha for prophylaxis and on-demand treatment of bleeding episodes in PTPs with severe hemophilia A. For on-demand treatment, 70.6% of infusions were rated either excellent or good [24].

Clinical trials in previously untreated patients

By September 1997, 101 severe PUPs were enrolled in a safety and efficacy study. In this study with ReFacto, 65% of bleeding episodes were resolved with a single infusion and 93% were resolved with one to three infusions. Forty surgical procedures in 30 patients were performed with excellent hemostasis [20].

Overall, 32 (31.7%) PUPs developed FVIII inhibitor antibodies after a median of 12 exposure days (range 3–49). Sixteen of these 32 patients had high-titer antibodies and 16 had low (<5 BU/mL) and/or transient titers [9]. The high-titer inhibitor rate using ReFacto was 15.8%, which is similar to the rate of the two full-length rFVIII concentrates [22].

Comments on inhibitor development with recombinant factor VIII concentrates

The results of several prospective trials of rFVIII in PUPs that were carefully monitored with laboratory inhibitor assays at regular 3-month intervals suggested that the incidence of inhibitor development in infants and children with severe

hemophilia A may be much higher than previously thought. The cumulative incidence in patients receiving rFVIII concentrates appeared to be in the order of 25%. A much lower incidence has been reported in patients receiving various types of pdFVIII concentrates, although figures vary from 10% to 52% [25,26].

Vermylen [27] elegantly reviewed the development of hemophilic inhibitors and indicated that there is probably enough evidence to suggest that repeated switching from one type of FVIII product to another facilitates a multifactorial immune response. In a more recent review, however, there was no evidence for increased inhibitor incidence in patients changing from pdFVIII to rFVIII [28]. It therefore remains unclear whether pdFVIII offers a protective effect against inhibitor development, perhaps by influencing immunomodulatory mechanisms of the immune system or by the blockade of epitopes by VWF. Furthermore, rFVIII may appear to potentiate the risk of developing inhibitors because this product is increasingly being used for continuous infusion, particularly in patients with moderate to mild disease who have previously had limited exposure to replacement therapy. It may also be pertinent in this context that intensive FVIII gene analysis in three rFVIII PUPs studies demonstrated that inhibitors were present more commonly in patients with large gene deletions, nonsense mutations, or the intron 22 inversion [29].

Comments on the development of antifactor VIII antibodies and antibodies against foreign proteins with recombinant factor VIII concentrates

In each clinical study using rFVIII concentrates, increases in antibodies to FVIII, CHO cell-/BHK cell-derived components, or mouse IgG measured by enzyme-linked immunosorbent assay (ELISA) were observed in some patients. The antibody development or increase in antibody levels (for FVIII, CHO, BHK, or mouse IgG) was not associated with clinical signs or symptoms and in no case did they prevent further treatment with each rFVIII concentrate. The clinical significance of these antibodies is unknown [30].

Future recombinant factor VIII concentrates with a prolonged half-life

New rFVIII products with prolonged half-lives are now intensively investigated and developing. Current technologies that extend the activity of FVIII include liposomal formulation and incorporation of high-molecular-weight polyethylene glycol (PEG) [31]. These would allow less frequent infusions and increase the effectiveness of hemophilia A prophylaxis through improved compliance [32].

Recombinant factor IX

The unique challenge in the development of rFIX protein for clinical use was the need for post-translational modifications, such as γ-carboxylation, sulfation, and propeptide cleavage. For its manufacturing process, the Genetics Institute uses a CHO cell line that has been cotransfected with an rFIX cDNA expression plasmid and a cDNA expression plasmid encoding an engineered form of the protease PACE (paired basic amino acid cleaving enzyme). PACE is necessary for the proper cleavage of the signal peptide and secretion of FIX [33].

Another challenge has been to develop a method to eliminate foreign proteins from the production process. Now all CHO cells used in the synthesis of rFIX are grown in serum-free medium containing only sucrose, amino acids, salts, rh-insulin, and vitamin K. Thus, a major advantage of the product, trade-named BeneFIX, is that it is virtually risk-free in terms of transmission of blood-borne viruses and spongiform agents [33].

Pharmacokinetic studies

A double-blind, randomized, crossover study was conducted in 11 patients with hemophilia B. The elimination half-lives of the rFIX and monoclonal antibody-purified pdFIX were 17.7 h and 18.1 h, respectively. *In vivo* recovery of rFIX, however, was 28% lower than that of pdFIX ($P < 0.05$). This difference was probably because of the difference in sulfation of Tyr155 and phosphorylation of Ser158, suggesting that these residues are important in the clearance of FIX. There was no evidence of increased thrombogenicity with rFIX in this study [33].

Clinical trials in previously treated patients

An open-label, multicenter study to evaluate the long-term safety, efficacy, and pharmacokinetics of rFIX concentrate has been performed. In 55 out of 56 patients, hemostatic efficacy was rated as excellent or good in the majority of bleeding episodes and surgical procedures. Eighty percent (854/1070) of new bleeding episodes were treated with a single infusion of rFIX [33]. One subject discontinued the study after 1 month of treatment because of bleeding episodes that were difficult to control. The subject's dose had not been adequately titrated.

Another patient developed a low-titer FIX inhibitor after 39 exposure days to rFIX. The peak titer was 1 BU/mL and the inhibitor disappeared after 11 months [33]. Clinical responses during 13 different surgical procedures ($n = 24$), including orthotropic liver transplantation, were rated as excellent or good in 97% of cases [34].

Clinical trials in previously untreated patients

By June 1999, 60 PUPs were enrolled in an open-label, multinational, multicenter study to evaluate safety and efficacy. Seventy-five percent of all bleeding episodes were managed with a single infusion of BeneFIX. In 50 PUPs with follow-up inhibitor assays, two developed high-titer inhibitors (maximum 42 and 18 BU/mL) with anaphylactoid reactions, which is well

known to be a unique feature of the FIX inhibitor response in 50% of patients on exposure to any FIX-containing product [35]. In view of the lower recovery values following rFIX infusion, dosage recommendations for BeneFIX are as follows:

Number of FIX units required = body weight (kg) × desired FIX increase (%) × 1.2

It should be noted that even lower recoveries may be seen in infants and young children [9].

Conclusion

rFVIII and rFIX preparations are now increasingly in clinical use in Europe, the USA, Canada, Japan, and elsewhere for patients with hemophilia. Clinical experience indicates that the incidence of inhibitors is probably not increased in PTPs or in PUPs. New second- and third-generation rFVIII or rFIX preparations, with no HSA as a stabilizer and with no animal/human proteins and/or murine immunoglobulin used in manufacture, are currently available commercially.

The greatest difficulty that has emerged with the increasing use of recombinant factor preparations for the treatment of hemophilia has been cost and exchange rate. These have led to a debate that has only been possible in the developed world [36].

The choice of product for replacement therapy must take into account three facts: (i) Plasma-derived factors are becoming ever safer; (ii) recombinant factors cost two to three times as much as plasma-derived factors; and (iii) the limited capacity to produce recombinant factors often causes periods of shortage [37]. In the UK and Italy, priority is given for the use of recombinant factors to PUPs and to patients without blood-borne infections despite previous exposure to plasma-derived factors [38].

Finally, as with any new technology, well-designed protocols and continued surveillance for any unexpected long-term complications are necessary.

Acknowledgment

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References

1 White GC, McMillan CW, Kingdon HS, Shoemaker CB. Use of recombinant antihemophilic factor in the treatment of two patients with classic hemophilia. *N Engl J Med* 1989; **320**: 166–70.
2 Schwartz RS, Abildgaard CF, Aledort LM, *et al.* Human recombinant DNA-derived antihemophilic factor (factor VIII) in the treatment of hemophilia A. *N Engl J Med* 1990; **323**: 1800–5.
3 Yoshioka A. Advances in hemophilia and other coagulation disorders; Recombinant factor VIII preparations. *Int J Pediatr Hematol Oncol* 1994; **1**: 491–7.
4 Lee DC, Millar JLA, Petteway SR, Jr. Pathogen safety of manufacturing process for biological products; special emphasis on Kogenate Bayer. *Haemophilia* 2002; **8**(Suppl. 2): 6–9.
5 Abshire TC, Brackmann H-H, Scharrer I, *et al.* Sucrose formulated recombinant human antihemophilic factor FVIII is safe and efficacious for treatment of hemophilia A in home therapy. *Thromb Haemost* 2000; **83**: 811–16.
6 Scharrer I, the Kogenate Bayer Study Group. Experience with Kogenate Bayer in surgical procedures. *Haemophilia* 2002; **8**(Suppl. 2): 15–18.
7 Musso R, Santagostino E, Faradji A, *et al.* Safety and efficacy of sucrose-formulated full-length recombinant factor VIII; experience in the standard clinical setting. *Thromb Haemost* 2006; **99**: 52–8.
8 Lusher JM, Arkin S, Abildgaard CF, Schwartz RS. Recombinant factor VIII for the treatment of previously untreated patients with hemophilia A. *N Engl J Med* 1993; **328**: 453–9.
9 Lusher JM. Recombinant clotting factors. A review of current clinical status. *Bio-Drugs* 2000; **13**: 289–98.
10 Giangrande PLF, for the Kogenate Bayer Study Group. Safety and efficacy of Kogenate Bayer in previously untreated patients (PUPs) and minimally treated patients (MTPs). *Haemophilia* 2002; **8**(Suppl. 2): 19–22.
11 Kreuz W, Gill JC, Rothschild C, *et al.* Full-length sucrose-formulated recombinant factor VIII for treatment of previously untreated or minimally treated young children with severe haemophilia A: Results of an international clinical investigation. *Thromb Haemost* 2005; **93**: 457–67.
12 Kaufman RJ, Wasley LC, Dorner AJ. Synthesis, processing and secretion of recombinant human factor VIII expressed in mammalian cells. *J Biol Chem* 1988; **263**: 6352–62.
13 Ewenstein BM, Collins P, Tarantino MD, *et al.* Hemophilia therapy innovation: Development of an advanced category recombinant FVIII by plasma/albumin-free method. *Semin Hematol* 2004; **41**(Suppl. 2): 1–18.
14 White GC, Courter S, Bray GL, *et al.* A multicenter study of recombinant factor VIII (Recombinate) in previously treated patients with hemophilia A. *Thromb Haemost* 1997; **77**: 660–7.
15 Blanchette VS, Shapiro AD, Liesner RJ, *et al.* Plasma and albumin-free recombinant factor VIII: Pharmacokinetics, efficacy and safety in previously treated pediatric patients. *J Thromb Haemost* 2008; **6**: 1319–26.
16 Negrier C, Shapiro A, Berntorp E, *et al.* Surgical evaluation of recombinant factor VIII prepared using a plasm/albumin-free method: Efficacy and safety of Advate in previously treated patients. *Thromb Haemost* 2008; **100**: 217–23.
17 Bray GL, Gomperts ED, Courter S, *et al.* A multicenter study of recombinant factor VIII (Recombinate): Safety, efficacy, and inhibitor risk in previously untreated patients with hemophilia A. *Blood* 1994; **83**: 2428–35.
18 Toole JT, Pittman DD, Orr EC, *et al.* A large region (≈95 kDa) of human factor VIII is dispensable for in vitro procoagulant activity. *Proc Natl Acad Sci USA* 1986; **83**: 5939–42.
19 Sandberg H, Almstedt A, Brandt J. Structural and functional characteristics of a B-domain deleted recombinant factor VIII molecule, r-VIIISQ. *Thromb Haemost* 2001; **85**: 93–100.
20 Date on file (Module 2.3.5 Drug Substance Manufacture, April 2007), Wyeth Research.
21 Mikaelsson M, Oswaldsson U, Jankowski MA. Measurement of factor VIII activity of B-domain deleted recombinant factor VIII. *Semin Hematol* 2001; **38**(Suppl. 4): 13–23.

22 Lusher JM, Lee CA, Kessler CM, Bedrosian CL-for the Refacto 3 Study Group. The safety and efficacy of B domain deleted recombinant factor VIII concentrate in patients with severe haemophilia A. *Haemophilia* 2003; **9**: 38–49.
23 Recht M, Abshire T, Lusher J, *et al.* Results of a single-dose randomized, double-blind, 2-period crossover pharmacokinetic study of b-domain deleted recombinant FVIII (BDDrFVIII), current manufacturing process (REFACTO) and BDDrFVIII, Albumin-free manufacturing process (REFACTO AF). *Blood* 2003; **102**: Abstract 2942.
24 Recht M, Nemes L, O'brein AC, *et al.* ReFacto AF is effective and safe in previously treated patients with hemophilia A: Final results of a pivotal phase III study. *Blood* 2007; **110**: Abstract 1151.
25 Scharrer I, Bray GL, Neurtzling O. Incidence of inhibitors in haemophilia A patients—a review of recent studies of recombinant and plasma-derived factor VIII concentrates. *Haemophilia* 1999; **5**: 145–54.
26 Goudemand J, Rothschild C, Demiguel V, *et al.* Influence of the type of factor VIII inhibitors in previously untreated patients with severe hemophilia A. *Blood* 2006; **107**: 46–51.
27 Vermylen J. How do some haemophiliacs develop inhibitors? *Haemophilia* 1998; **4**: 538–42.
28 Scharrer I, Ehrlich HJ. Lack of evidence for increased inhibitor incidence in patients switched from plasma-derived to recombinant factor VIII. *Haemophilia* 2001; **7**: 346–8.
29 Williams IJ, Peake IR, Goodeve AV. Recombinant PUP mutation study: Relationship between factor VIII mutation and inhibitor development. *Haemophilia* 1998; **4**: 228.
30 Fukui H, Yoshioka A, Shima M, *et al.* Clinical evaluation of recombinant human factor VIII (BAY w 6240) in the treatment of hemophilia A. *Int J Hematol* 1991; **54**: 419–27.
31 Baru M, Carmel-Goren L, Barenholz Y, *et al.* Factor VIII efficient and specific non-covalent binding to PEGylated liposomes enables prolongation of its circulation time and haemostic efficacy. *Thromb Haemost* 2005; **93**: 1061–8.
32 Spira J, Plyushch OP, Andreeva TA, Andreev Y. Prolonged bleeding-free period following prophylactic infusion of recombinant factor VIII reconstituted with pegylated liposomes. *Blood* 2006; **108**: 3668–13.
33 White GC, Beebe A, Nielsen B. Recombinant factor IX. *Thromb Haemost* 1997; **78**: 261–5.
34 White GC, Shapiro A, Ragni M, *et al.* Clinical evaluation of recombinant factor IX. *Semin Hematol* 1998; **35**(Suppl. 2): 33–8.
35 Warrier I. Factor IX inhibitor and anaphylaxis. In: Rodriguez-Merchan EC, Lee CA (eds.) *Inhibitors in Patients with Haemophilia*. Oxford: Blackwell Science, 2002: 87–91.
36 Lee C. (1999) Recombinant clotting factors in the treatment of hemophilia. *Thromb Haemost* 1999; **82**: 516–24.
37 Mannucci PM, Tuddenham EGD. The hemophiliacs—from royal genes to gene therapy. *N Engl J Med* 2001; **344**: 1773–9.
38 United Kingdom Haemophilia Centre Doctors' Organization (UKHCDO). Guidelines on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders. *Haemophilia* 2003; **9**: 1–23.

23 Plasma-derived coagulation factor concentrates

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Introduction

The development of blood products for the treatment of hemophilia has dramatically altered the prognosis for those patients who live in affluent countries and have regular access to safe products. The median life expectancy for people with severe hemophilia increased fivefold from only 11 years during the period 1831–1920 to 56.8 years during the period 1961–80 [1]. In more recent years, infection with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) has had a significant negative impact. Access to treatment also improves the quality of life of patients, at least in part by facilitating access to normal education and employment [2]. In recent years, the relative merits of plasma versus recombinant products have been a major topic of debate. The arguments focus primarily on safety with regard to transmission of pathogens, which must be of prime concern in the selection of products for the treatment of hemophilia. However, the relative immunogenicity of the two classes of product has also been a subject of controversy. The recent experience of an acute and worldwide shortage of recombinant factor VIII has certainly served to focus minds on the fact that the number of manufacturing plants is very limited, particularly so in the cases of recombinant factor IX and recombinant factor VIIa. However, one important positive consequence of the progressive switch to recombinant products in developed countries is that this will help to secure effective and safe treatment for people in developing countries. As patients in more affluent parts of the world such as North America, Europe, Australia, and Japan convert inexorably to recombinant products, manufacturers of plasma-derived products will be forced to seek new markets in the developing world and these will also have to be competitively priced. It is clear that there will continue to be a global requirement for plasma-derived as well recombinant coagulation factor concentrates for many years to come.

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Cryoprecipitate

Although coagulation factor concentrates are now regarded as the treatment of choice for hemophilia in developed countries, it must be recognized that cryoprecipitate continues to form the mainstay of treatment for patients in many less affluent countries around the world. The discovery by Judith Pool in 1964 that a fraction of thawed plasma contained factor VIII was a major landmark in the development of products for the treatment of hemophilia. Cryoprecipitate is prepared by slow thawing of fresh-frozen plasma (FFP) at 4°C for 24 h, when cryoprecipitate appears as an insoluble precipitate and separated by centrifugation. It contains significant quantities of factor VIII, von Willebrand factor (VWF), fibrinogen and factor XIII (but not factor IX or XI). Current AABB (American Association of Blood Banks) standards call for a minimum standard of 80 international units (IU) factor VIIII per pack (and 150 mg fibrinogen). However, in practice the coagulation factor content of individual packs in developing countries is variable and is usually not controlled. The yield of factor VIII from plasma can be enhanced by controlling several variables, and in one study, packs with a factor VIII content of 150 IU or more were produced using an automated device with computer-controlled temperature cycling [3]. However, the most significant problem with cryoprecipitate is that it cannot be easily subjected to viral inactivation procedures (such as heat or solvent/detergent treatment) and this inevitably translates into a risk of transmission of viral pathogens, which is not insignificant with repeated exposure. For example, a study based on data from Venezuela estimated a cumulative risk of 40% for HIV and almost 100% for HCV over a lifetime (60 years) of treatment with cryoprecipitate [4]. The use of this product in the treatment of congenital bleeding disorders, therefore, cannot be recommended in countries which can afford coagulation factor concentrates. Certain steps can at least be taken to minimize the risk of transmission of viral pathogens. These include careful selection of donors and producing packs from single donors. Once collected, the plasma should be quarantined until the donor has been recalled and retested for markers of infection: if the donor does not return, the plasma should not be used. Polymerase

chain reaction (PCR) testing is a technology which has a potentially much greater relevance for the production of cryoprecipitate than concentrates, as the latter are subjected to viral inactivation steps. Quality control, involving the monitoring of factor VIII content, is also very important. A method for solvent/detergent treatment of cryoprecipitate has recently been developed for application in the developing world, which should help to make this product safer [5].

Much of what has been written about cryoprecipitate applies to the use of FFP, which is a source of all coagulation factors. As it contains factor IX, it is still used for the treatment of hemophilia B in countries unable to afford the use of plasma-derived factor IX concentrate. Packs of FFP subjected to some form of virucidal treatment (including solvent/detergent treatment) are already available. The possibility of severe allergic reactions to infused plasma, including TRALI (transfusion-related acute lung injury) attributed to cytotoxic antibodies of donor origin in the infused plasma, have been recognized for some time [6,7]. An additional benefit of solvent/detergent-treated FFP is a significant decrease in the incidence of such allergic reactions [8].

Principles of manufacture

There are some common steps involved in the manufacture of coagulation factor concentrates. Plasma proteins such as albumin, coagulation factor concentrates, and immune globulin preparations are manufactured from large pools of human plasma, primarily by the Cohn cold ethanol fractionation method. This method, developed by Edwin Cohn in Boston in the 1940s, involves the sequential precipitation of specific proteins under varying conditions of ethanol and pH conditions.

In the case of factor VIII, cryoprecipitate is produced using a standard ethanol/dry ice process for snap freezing and the cryoprecipitate is extracted by thawing in a 4°C. Antihemophilic factor (AHF) is extracted from the cryoprecipitate by dissolving in a buffer. Fibrinogen is removed from the resulting supernatant by precipitation, followed by precipitation of factor VIII from supernatant. Factor VIII is then purified by chromatographic techniques, either using ion-exchange chromatography or immunoaffinity chromatography.

Factor IX is prepared by anion-exchange chromatography in the presence of heparin, applied to cryoprecipitate-depleted plasma, or the use of immunoaffinity chromatography.

The coagulation protein (factor VIII or IX) is then freeze dried and lyophilized concentrate bottled under sterile conditions. At some stage, either as a final step or during the manufacturing process, a specific virucidal step such as heat treatment and/or solvent/detergent treatment is applied (see below).

Quality control is an essential element in the manufacturing process, and each batch of product is randomly sampled and analyzed for factor VIII (or IX) clotting activity, electrolyte concentration, pyrogenicity, sterility, and toxicity. If found to comply with all release parameters, the bottles are labeled with a batch number and bottle number before being issued for use. Regulatory agencies such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) generally conduct some form of independent oversight of this process by routine monitoring of the manufacturer's test results or by conducting their own tests.

The plasma used for fractionation may be recovered plasma, typically derived from whole blood procured from volunteer donors, or source plasma, usually collected from paid donors who undergo periodic plasmapheresis. In the past, plasma for fractionation from paid donors was considered to be at higher risk of viral infection than plasma from voluntary donors drawn from the same population. However, this can no longer be considered to be the case. Donor selection procedures are designed to identify and exclude donors at risk of being infected with pathogenic viruses. Exclusion criteria include a history of blood-borne infection, intravenous drug use, and high-risk sexual behavior. The quarantining of plasma until a donor appears for retesting (inventory hold) is an additional precaution which may be taken. Nucleic acid testing (NAT) is now routinely employed by an increasing number of manufacturers for detection of a wide range of viruses including HIV, HCV, parvovirus B19, and both hepatitis A and B.

The establishment of a "plasma master file" for plasma-derived products is a concept which has been pioneered by the European regulatory authorities (including the EMEA). This contains details of all donations in a batch of products. This permits tracing of blood donations through the screening procedure right up to intravenous administration. Within Europe, the plasma master file replaces that part of the marketing authorization application (MAA) describing the raw material plasma and makes the arrangements for movement of plasma, intermediates, and products across member states both easier and more transparent. Two particular issues deserve further consideration: purity of product and the number of virucidal steps.

Product purity

Product purity should not be confused with concentrate safety or efficacy. Purity simply refers to the percentage of the desired ingredient (e.g., factor VIII) in concentrates relative to other ingredients present. Concentrates on the market vary widely in their purity from around 5.0 IU factor VIII/mg protein in intermediate-purity concentrates to 2000 in the case of high-purity concentrates. Generally, products which are produced at higher purity tend to be associated with low manufacturing yields and therefore cost more. High-purity products are more readily soluble, which is more convenient for home treatment and also facilitates administration by continuous infusion if desired in the setting of surgery. The incidence of allergic

reactions is also probably lower with high-purity products. However, there is no clear evidence that modern high-purity concentrates offer a higher margin of safety with regard to transmission of pathogens. Several studies have suggested that the use of high-purity concentrates retards the decline in $CD4^+$ lymphocyte counts in HIV individuals, but this has not been a consistent finding [9,10]. However, it has not been clearly demonstrated that any resulting change in $CD4^+$ lymphocyte numbers is associated with a slowing in the rate of progression to acquired immune deficiency syndrome (AIDS) or death and any such positive effect of high-purity products is insignificant when compared with the immune reconstitution associated with highly active antiretroviral therapy (HAART).

One advantage of less pure factor VIII concentrates products is that they usually contain significant quantities of VWF so that they may be useful in the treatment of that condition. Examples of concentrates suitable for the treatment of von Willebrand disease include Optivate® and 8Y® (BPL), Alphanate® and Fanhdi® (Grifols), Haemate P® (CSL Behring), and Wilfactin® (LFB) [11]. None of the brands of plasma-derived high-purity factor VIII or recombinant concentrates contain VWF.

The relative immunogenicity of recombinant and plasma-derived factor VIII concentrates has been a source of recent debate and controversy. It is clear that the principal determinant of inhibitor development is the underlying molecular abnormality, with large deletions and nonsense mutations being associated with a particularly high risk. However, an early systematic review of cohort studies and registry data published found that patients who used a single plasma-derived factor VIII preparation had a lower inhibitor incidence than those treated with multiple plasma-derived concentrates or a single full-length recombinant product [12]. The results of four observational studies are now available which compare inhibitor development among recipients of recombinant and plasma-derived products [13–16]. Despite methodological differences, the results of these four studies seem to suggest a definite trend with an increased incidence of inhibitor development associated with recombinant factor VIII products [17]. It has been postulated that the large VWF protein may protect against inhibitor development by masking important epitopes in the amino-terminal of the light chain corresponding to the A3 domain and in the carboxy-terminus of the C2 domain of the factor VIII molecule [18–20]. *In vitro* data also indicate that VWF can prevent factor VIII from being endocytosed by dendritic cells and presented to T lymphocytes, thus resulting in lower immunogenicity [21].

It is clear that there is a need for further clinical trials to try and secure definitive answers to the questions raised by these studies. An international study is expected to be launched soon with the primary objective of comparing the immunogenicity of the two classes of products [22]. The SIPPET study is a multicenter, prospective, controlled, randomized, open-label clinical trial on inhibitor frequency in previously untreated patients (PUPs). A related issue which needs further attention is whether plasma-derived products are associated with a better response to immune tolerance in subjects who develop antibodies, as has been suggested by some groups [23].

In the case of factor IX concentrates, high-purity concentrates have been shown to induce less activation of coagulation than prothrombin complex concentrates [24]. The latter should no longer be employed in the routine management of hemophilia B in view of case reports of thrombosis (including venous thromboembolism, disseminated intravascular coagulation, and myocardial infarction) associated with their use [25–27]. There is no suggestion of an increased risk of inhibitor development associated with recombinant factor IX compared with that seen with plasma-derived factor IX [28].

Methods of viral inactivation and elimination

The introduction of heat treatment and solvent/detergent treatment using such agents as TNBP and Triton X-100 in the mid-1980s effectively eliminated the risk of transmission of HIV and HCV through the use of plasma-derived products [29,30]. It has since proved to be highly effective against a wide range of newly emerged viral pathogens with a lipid envelope such as West Nile virus, the severe acute respiratory syndrome (SARS) coronavirus, and avian influenza [31–33]. However, solvent/detergent treatment with such agents as TNBP and Triton X-100 does not inactivate nonenveloped viruses such as hepatitis A [34,35]. Furthermore, some viruses (such as human parvovirus B19) are relatively resistant to both types of physical process [36,37]. Whilst infection with parvovirus is rarely of clinical significance, it is naturally of concern that this hardy DNA virus is resistant to physical virucidal treatments.

There are no screening tests available for the detection of prions, including the presumed causative agent of variant Creutzfeldt–Jakob disease (vCJD), and precautions are largely based on donor exclusion. All regulatory authorities have regulations in place to exclude donors who have spent defined periods in countries considered to be at high risk of bovine spongiform encephalopathy (BSE) or vCJD, such as the UK. Furthermore, these pathogenic protein particles are completely resistant to conventional heat and solvent/detergent treatment. It is therefore reassuring that plasma fractionation techniques appear quite fortuitously to eliminate substantial amounts of prions [38]. By contrast, nanofiltration is also highly effective in eliminating prions in experimental conditions. Nanofiltration was originally developed as an alternative method of removing a wide range of viruses, including parvovirus B19. It is a relatively simple manufacturing step that consists of filtering protein solution through membranes of a very small pore size, typically 15–40 nm, under conditions that retain viruses by a mechanism largely based on size exclusion. A variety of filters have now been developed by commercial companies for

removal of prions from blood and plasma, which incorporate ligands with affinity for prion proteins [39]. No cases of overt CJD have ever been reported in a subject with hemophilia. However, prions were identified post mortem in the spleen of one elderly hemophilic man in the UK who had no neurologic problems and who died of unrelated causes.

Nanofiltration has also been demonstrated not to induce protein alteration (e.g., neoantigenicity), and the yield of filtered protein is not adversely affected. Nanofiltration of factor VIII products is also possible nowadays, despite a relatively large molecular weight of 330 kDa compared with 60 kDa for factor IX [40]. The Planova® filtration device is particularly suitable for factor VIII preparations and is composed of hollow-fiber microporous membranes made of naturally hydrophilic cuprammonium-regenerated cellulose, housed in a polycarbonate body. It is an efficient method of removing more than four to six logs of a wide range of viruses, and has the added advantage of having no adverse, denaturing effect on plasma proteins.

All virus inactivation and removal steps have their limitations. It is recommended that two distinct and effective steps that are complementary be incorporated into the plasma product manufacturing process [11]. European guidelines recommend that at least one step effectively inactivates or removes nonenveloped viruses. A 2001 recommendation from the Committee for Proprietary Medicinal Products (CPMP) states that:

for all plasma-derived medicinal products, it is an objective to incorporate effective steps for inactivation/removal of a wide range of viruses of diverse physicochemical characteristics. In order to achieve this, it will be desirable in many cases to incorporate two distinct effective steps which complement each other in their mode of action such that any virus surviving the first step would be effectively inactivated/removed by the second. At least one of the steps should be effective against nonenveloped viruses. Where a process step is shown to be reliably effective in inactivating or removing a wide range of viruses including enveloped and nonenveloped viruses of diverse physicochemical characteristics and the process contains additional stages reliably contributing to the inactivation/removal of viruses, a second effective step would not be required [11].

It is recommended that all patients receiving plasma-derived concentrates be vaccinated against hepatitis A and B as an additional precaution [41].

Potency and labeling issues

Assays of coagulation factors have been standardized by the establishment of international standards by the World Health Organization (WHO). These standards define the IU and are available in limited quantities for calibration of local, commercial, national, and supranational standards—these in turn are used to assay therapeutic concentrates and plasma samples from patients and hence all such measurements can be recorded in IU [42]. When the first international standards for each coagulation factor were established, they were calibrated against fresh normal plasma from a large number of donors, and so 1 IU is approximately equivalent to the amount of each factor in 1 ml of average normal plasma. An important principle in biologic standardization is that of "like versus like." For many biologic substances, not just coagulation factors, reproducibility between laboratories, and between assay methods, is greatest when test and standard are of similar composition. It has been found in several collaborative studies that plasma standards are unsuitable for assay of coagulation factor concentrates and vice versa. There are therefore two WHO standards for all the principal coagulation factors: one for the assay of therapeutic concentrates and the other for assay of plasma samples.

The current (seventh) WHO factor VIII standard for concentrates was prepared from a plasma-derived factor VIII concentrate containing VWF and subjected to solvent/detergent treatment [43]. Other standards are the US Mega and European Pharmacopoeia (EP) standards, which are working standards calibrated against the WHO standard in multicenter studies. The current US Mega (2) and EP (3) standards are identical, shared from the same large batch of a plasma-derived concentrate. In the USA, most manufacturers use the Mega standard to assay their product, whereas most manufacturers in Europe use an internal house standard calibrated against the WHO standard. All three assay methods are still in use, although the two-stage method is used by only a few manufacturers. Most US manufacturers use the one-stage method, although most European manufacturers of plasma-derived concentrates now use the chromogenic method, which is the recommended method of both the EP and the International Society on Thrombosis and Haemostasis. In the case of plasma-derived products, significant discrepancies are typically observed between the results of one-stage and chromogenic assays found with the "Method M" products; the former giving potencies around 25–30% higher than those obtained with the chromogenic method [44].

Selection of products

The World Federation of Hemophilia (WFH) has published a guide [45] for the assessment of clotting factor concentrates, which deals with all types of available products but focuses primarily on plasma-derived products. The UK Haemophilia Centre Doctors' Organization (UKHCDO) has also published relevant guidelines [11]. It is beyond the scope of this chapter to include details of all the available coagulation factor concentrates, but the WFH guide also includes a registry of all coagulation factor concentrates. This is updated on an annual basis and is also available through the WFH web site (www.wfh.org). The registry includes information on donors (nationality, whether paid or voluntary); method of obtaining plasma;

serologic tests on donors; testing of mini-pools for viruses using PCR amplification; location of fractionation facilities; methods of fractionation; methods of viral inactivation/elimination; levels of purification; identity of distributor and manufacturer; and intended area of distribution (domestic or export).

Consideration needs to be given to both the plasma source as well as the manufacturing process. Cost alone should certainly not be the deciding factor when choosing products. As regards the plasma source, the plasma supplier should be licensed by the relevant national health authority and the donor epidemiology scrutinized. At the very least, blood testing should include screening of individual donations (not just mini-pools of plasma) for HIV, HBV, and HCV, preferably using NAT technology. Evidence of a robust quality assurance system for the performance of viral screening tests is vital. Products should be subjected to well-validated viral inactivation/removal steps. Data relating to stability data and shelf-life may also be important. Clinical reports of previous use are also important, including details of where the product is currently available and used in clinical practice and marketing authorizations from licensing bodies. A product license from organizations such as the FDA and EMEA certainly implies that the product has been subject to a high degree of scrutiny. Published data in peer-reviewed journals, covering areas such as adverse events and efficacy, are also important.

Plasma-derived concentrates for rare bleeding disorders

Plasma-derived concentrates of fibrinogen, factor VII, factor XI, and factor XIII are available [46]. Clinical data on the use of such concentrates may be limited and not all have product licenses and thus need to be used on a named-patient basis. All prothrombin complex concentrates contain factor II (prothrombin), factor IX, and factor X; some also contain factor VII. The products containing four coagulation factors are widely used to reverse the effect of anticoagulation. They may also be used for the treatment of isolated congenital deficiencies of these factors, but it should be noted that the potency is usually assigned to the vials according to the factor IX content and the amount of the other factors may be quite different. Recombinant activated factor VII is now regarded as the treatment of choice for congenital factor VII deficiency. No concentrate containing factor V is available and treatment with fresh-frozen plasma is required in such cases.

References

1 Larsson SA. Life expectancy of Swedish haemophiliacs, 1831–1980. *Br J Haematol* 1985; **59**: 593–602.

2 Royal S, Schramm W, Berntorp E, *et al.* Quality-of-life differences between prophylactic and on-demand factor replacement therapy in European haemophilia patients. *Haemophilia* 2002; **8**: 44–50.

3 Rock G, Berger R, Lange J, Tokessy M, Palmer DS, Giulivi A. A novel, automated method of temperature cycling to produce cryoprecipitate. *Transfusion* 2001; **41**: 232–5.

4 Evatt BL, Austin H, Leon G, Ruiz-Saez A, De Bosch N. Haemophilia therapy: Assessing the cumulative risk of HIV exposure by cryoprecipitate. *Haemophilia* 1999; **5**: 295–300.

5 Burnouf T, Caron C, Radosevich M, Goubran HA, Goudemand J, El-Ekiaby M. Properties of a concentrated minipool solvent-detergent treated cryoprecipitate processed in single-bag systems. *Haemophilia* 2008; **14**: 956–62.

6 Kernoff PB, Durrant IJ, Rizza CR, Wright FB. Severe allergic pulmonary oedema after plasma transfusion. *Br J Haematol* 1972; **23**: 777–81.

7 Bux J. Transfusion-related acute lung injury (TRALI): A serious adverse event of blood transfusion. *Vox Sanguinis* 2005; **89**: 1–10.

8 Riedler GF, Haycox AR, Duggan AK, Dakin HA. Cost-effectiveness of solvent/detergent-treated fresh-frozen plasma. *Vox Sanguinis* 2003; **85**: 88–95.

9 Goedert JJ, Cohen AR, Kessler CM. Risks of immunodeficiency, AIDS and death related to purity of factor VIII concentrate. Multicenter Hemophilia Cohort Study. *Lancet* 1994; **344**: 791–2.

10 Sabin C, Pasi J, Phillips A, Elford J, Janossy G, Lee CA. CD4+ counts before and after switching to monoclonally high-purity factor VIII concentrate in HIV-infected haemophilic patients. *Thromb Haemost* 1994; **72**: 214–17.

11 Keeling D, Tait C, Makris M, on behalf of United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO). Guideline on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders. *Haemophilia* 2008; **14**: 671–84.

12 Wight J, Paisley S. The epidemiology of inhibitors in haemophilia A: A systematic review. *Haemophilia* 2003; **9**: 418–35.

13 Goudemand J, Rothschild C, Demiguel V, *et al.* Influence of the type of factor VIII concentrate on the incidence of factor VIII inhibitors in previously untreated patients with severe hemophilia A. *Blood* 2006; **107**: 46–51.

14 Gouw SC, van der Bom JG, Auerswald G, Ettinghausen CE, Tedgard U, van den Berg HM. Recombinant versus plasma-derived factor VIII products and the development of inhibitors in previously untreated patients with severe hemophilia A: The CANAL cohort study. *Blood* 2007; **109**: 4693–7.

15 Chalmers EA, Brown SA, Keeling D, *et al.* Early factor VIII exposure and subsequent inhibitor development in children with severe haemophilia A. *Haemophilia* 2007; **13**: 149–55.

16 Escurolia-Ettingshausen C, Kreuz W. Recombinant vs. plasma-derived products, especially those with intact VWF, regarding inhibitor development. *Haemophilia* 2006; **12**(Suppl. 6): 102–6.

17 Calvez T, Laurian Y, Goudemand J. Inhibitor incidence with recombinant vs. plasma-derived FVIII in previously untreated patients with severe hemophilia A: Homogenous results from four published observational studies. *J Thromb Haemost* 2008; **6**: 390–2.

18 Kallas A, Talpsep T. von Willebrand factor in factor VIII concentrates protects against neutralization by factor VIII antibodies of haemophilia A patients. *Haemophilia* 2001; **7**: 375–80.

19 Suzuki T, Arai M, Amano K, Kagawa K, Fukutake K. Factor VIII inhibitor antibodies with C2 domain specificity are less inhibitory to factor VIII complexed with von Willebrand factor. *Thromb Haemost* 1996; **76**: 749–54.

20 Shima M, Nakai H, Scandella D, *et al.* Common inhibitory effects of human anti-C2 domain inhibitor alloantibodies on factor VIII binding to von Willebrand factor. *Br J Haematol* 1995; **91**: 714–21.
21 Dasgupta S, Repessé Y, Bayry J, *et al.* VWF protects FVIII from endocytosis by dendritic cells and subsequent presentation to immune effectors. *Blood* 2007; **109**: 610–12.
22 Mannucci PM, Gringeri A, Peyvandi F, Santagostino E. Factor VIII products and inhibitor development: The SIPPET study (Survey of Inhibitors in Plasma-Product Exposed Toddlers). *Haemophilia* 2007; **13**(Suppl. 5): 65–8.
23 Kurth MAH, Dimichele D, Sexauer C, Sanders JM, Torres M, Zappa SC, Ragni M, Leonard N. Immune tolerance therapy utilizing factor VIII/von Willebrand factor concentrate in haemophilia A patients with high titre factor VIII inhibitors. *Haemophilia* 2008; **14**: 50–5.
24 Thomas DP, Hampton KK, Dasani H, *et al.* A cross-over pharmacokinetic and thrombogenicity study of a prothrombin complex concentrate and a purified factor IX concentrate. *Br J Haematol* 1994; **87**: 782–8.
25 Kasper CK. Thromboembolic complications. *Thrombosis et Diathesis Haemorrhagica* 1975; **33**: 640–4.
26 Conlan MG, Hoots WK. Disseminated intravascular coagulation and hemorrhage in hemophilia B following elective surgery. *Am J Hematol* 1990; **35**: 203–7.
27 Fuerth JH, Mahrer P. Myocardial infarction after factor IX therapy. *JAMA* 1981; **245**: 1455–6.
28 Poon MC, Lillicrap D, Hensman C, Card R, Scully MF. Recombinant factor IX recovery and inhibitor safety: A Canadian post-licensure surveillance study. *Thromb Haemost* 2002; **87**: 431–5.
29 Horowitz MS, Rooks C, Horowitz B, Hilgartner MW. Virus safety of solvent/detergent-treated antihaemophilic factor concentrate. *Lancet* 1988; **ii**: 186–9.
30 Schimpf K, Brackmann HH, Kreuz W, *et al.* Absence of anti-human immunodeficiency virus types 1 and 2 seroconversion after the treatment of hemophilia A or von Willebrand's disease with pasteurized factor VIII concentrate. *N Engl J Med* 1989; **321**: 1148–52.
31 Jakubik JJ, Vicik SM, Tannatt MM, Kelley BD. West Nile virus inactivation by the solvent/detergent steps of the second and third generation manufacturing steps for B-domain deleted recombinant factor VIII. *Haemophilia* 2004; **10**: 69–74.
32 Rabenau HF, Biesert L, Schmidt T, Bauer G, Cinatl J, Doerr HW. SARS-coronavirus (SARS-CoV) and the safety of a solvent/detergent (S/D) treated immunoglobulin preparation. *Biologicals* 2005; **33**: 95–99.
33 Jorquera JI. Safety procedures of coagulation factors. *Haemophilia* 2007; **13**(Suppl. 5): 41–6.
34 Vermylen J, Peerlinck K. Review of the hepatitis A epidemics in hemophiliacs in Europe. *Vox Sanguinis* 1994; **67**(Suppl. 4): 8–11.
35 Soucie JM, Roberston BH, Bell BP, McCaustland KA, Evatt BL. Hepatitis A virus infections associated with clotting factor concentrate in the United States. *Transfusion* 1998; **38**: 573–9.
36 Yee TT, Cohen BJ, Pasi KJ, Lee CA. Transmission of symptomatic parvovirus parvovirus B19 infection by clotting factor concentrate. *Br J Haematol* 1996; **93**: 457–9.
37 Azzi A, Morfini M, Mannucci PM. The transfusion-associated transmission of parvovirus B19. *Transfusion Medicine Reviews* 1999; **13**: 194–204.
38 Farrugia A. Risk of variant Creutzfeldt–Jakob disease from factor concentrates: Current perspectives. *Haemophilia* 2002; **8**: 230–5.
39 Prowse C. Prion removal with filters. *ISBT Science Series* 2006; **1**: 230–4.
40 Burnouf T, Radosevich M. Nanofiltration of plasma-derived biopharmaceutical products. *Haemophilia* 2003; **9**: 24–37.
41 Makris M, Conlon CP, Watson HG. Immunization of patients with bleeding disorders. *Haemophilia* 2003; **9**: 541–6.
42 Hubbard AR. International biological standards for coagulation factors and inhibitors. *Semin Thromb Hemost* 2007; **33**: 283–9.
43 Raut S, Bevan S, Hubbard AR, Sands D, Barrowcliffe TW. A collaborative study to establish the 7th International Standard for Factor VIII Concentrate. *J Thromb Haemost* 2005; **3**: 119–26.
44 Lee CA, Owens D, Bray G, *et al.* Pharmacokinetics of recombinant factor VIII (Recombinate) using one-stage clotting and chromogenic factor VIII assay. *Thromb Haemost* 1999; **82**: 1644–7.
45 Farrugia A. Guide for the assessment of clotting factor concentrates for the treatment of hemophilia. World Federation of Hemophilia (Montreal), 2003. Also available as a PDF file at www.wfh.org
46 Bolton-Maggs PH, Perry DJ, Chalmers EA, *et al.* The rare coagulation disorders—review with guidelines from the United Kingdom Haemophilia Centre Doctors' Organisation. *Haemophilia* 2004; **10**: 593–628.

24 Products used to treat hemophilia: recombinant factor VIIa

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Background

Recombinant factor VIIa (rFVIIa) was developed for treatment of acute bleeding in hemophilia patients with inhibitors against factor VIII (FVIII) or factor IX (FIX). Treatment of hemophilia patients with inhibitors remains a significant problem and includes treatment of acute bleeding as well as methods to induce immunologic tolerance in order to permanently eradicate the inhibitors.

Since the 1970s, prothrombin complex concentrates and activated prothrombin complex concentrates (aPCCs) have been used to treat hemophilia patients with inhibitors against FVIII or FIX. These concentrates contain all the vitamin K-dependent coagulation proteins, active forms of FIX, FVII, and FX, and trace amounts of FVIII protein. The use of aPCCs has, however, been associated with a certain risk of the development of thromboembolic side-effects, and the hemostatic effect in moderate to mild joint bleeds was only 50–60% in the three controlled studies reported, the latest published in 2008 [1]. One of the components in the aPCCs is activated FVII (FVIIa), which is the only activated coagulation protein that is not enzymatically active by itself. The requirement of tissue factor (TF) for FVIIa to become an active enzyme suggested that rFVIIa should be hemostatically active only at a local level, where TF is available. Observations in the 1980s indicated that pharmacologic doses of FVIIa also bound to pre-activated platelets localized at the site of injury [2]. Using rFVIIa alone therefore should minimize the risk of inducing a systemic activation of the coagulation system and thereby the risk of systemic thromboembolic side-effects.

Mechanism of action of recombinant factor VIIa

Normal hemostasis is initiated by the complex formation between TF and FVII or FVIIa. TF is expressed in a number of cells located in the deeper layers of the vessel wall and is exposed to the circulating blood only as a result of injury to the vessel wall [3]. TF may also originate from the circulating blood in the form of encrypted TF carried by cell elements [4]. Already in the 1970s, it was reported that platelet and platelet membranes enhanced the procoagulant activity of leukocytes [5]. A hemostatic effect of platelet membrane vesicles was also demonstrated in thrombocytopenic rabbits in 1987 [6]. It was reported recently that washed platelets incubated with TF were able to take up TF in a process involving traffic of vesicles through channels of the open canalicular system (OCS). TF was identified in the OCS and occasionally in the α-granulae of the platelets [7]. Whether platelet-related TF is constitutively present in platelets or transferred from other cells is the subject of debate [2]. In normal conditions the TF found on cell surfaces as well as in microvesicles is encrypted, which thus allows the circulation of such TF-containing particles to occur without generalized coagulation [2]. There is still no consensus on how the encrypted TF is decrypted, although the cell membrane composition seems to be of major importance [2]. It was recently reported that cultured mesothelial and endothelial cells increased their surface TF activity following exposure of thrombin or plasmin [8]. Recently, the presence of an alternatively spliced human TF that is soluble, circulates in blood, and exhibits procoagulant activity when exposed to phospholipids was reported. This soluble TF has also been found to be incorporated into thrombi [9]. Although there are reports on the presence of TF-containing microvesicles in various situations [10], the physiologic significance of these is still not clear.

The TF–FVII/FVIIa complexes formed following an injury to the vessel wall are localized to the site of injury because TF is a receptor protein anchored to cells by a transmembrane domain. The presence of a few functional FVIIa–TF complexes extravascularly was suggested and supports a continuous TF-dependent extravascular generation of small amounts of FIXa and FXa [3]. In fact, it was recently demonstrated that FVIIa is bound to perivascular TF in the absence of a clear injury [2]. Such complexes may mediate the activation of the hemostatic mechanism demonstrated in normal individuals during normal circumstances, resulting in the

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formation of the normally occurring FIX, FX, and prothrombin activation peptides [10]. The TF–FVIIa complexes activate FX into FXa, which converts a limited amount of prothrombin into thrombin sufficient to activate FVIII and FV as well as FXI and platelets. The FXa activity is restricted to the TF-bearing cell surface. Any FXa that diffuses off the cell is immediately inhibited by the tissue factor pathway inhibitor (TFPI) and antithrombin (AT). As soon as FXa is formed, a complex including TF–FVIIa and FXa is formed, inhibited by TFPI, and internalized. Most of the internalized FVIIa is degraded, but a small fraction recycles back to the cell surface as an intact protein [2]. The small amount of initial thrombin binds to platelets that have adhered to extravascular matrix components at the site of injury partially mediated by binding of von Willebrand factor to collagen. Thrombin enhances platelet activation, leading to release of FV as well as activation of FV and FVIII. The platelets activated by the initially formed thrombin expose negatively charged phospholipids on their surface, which enhances the binding of the activated coagulation proteins to the platelet surface. FIXa activated initially by the FVIIa–TF complex diffuses to the activated platelet and binds strongly to the negatively charged platelet surface where the most effective FX activation and thrombin generation take place [2,11]. The binding of coagulation proteins on the platelet surface is facilitated by the combined stimulation of the platelet collagen receptor (GPVI) and thrombin receptor owing to the development of a subpopulation of platelets with an increased binding capacity [2]. Individual variations in such subpopulations may add to the variability of platelet procoagulant response. The binding of FXIa seems also to be mediated by specific sites promoting the formation of active FIXa–FVIIIa ("tenase complex") [2]. The tenase complex activates FX from the circulation into FXa on the platelet surface, associates with FVa, and generates a burst of thrombin required to form a firm, well-structured fibrin hemostatic plug. The FX activation occurs quickly and only small amounts of FX are necessary for a saturated formation of FXa according to results obtained in a cell-based *in vitro* model [2]. FXI generated by the initial thrombin activates more FIX into FIXa on the platelet surface, thereby enhancing thrombin generation. All these reactions seem to be well regulated in terms of saturation of the activation processes. However, adding more prothrombin increases the thrombin generation without reaching any level of saturation [2]. The gel network formed at the gelpoint (clotting time) has been found to be important for the scaffold into which the subsequently activated fibrinogen molecules are incorporated, the primary scaffold becoming more porous the lower the thrombin concentration [2]. A tight fibrin network makes the hemostatic plug more resistant against premature proteolysis thereby helping to maintain hemostasis [2]. Also, a full thrombin burst is necessary for a full activation of the thrombin-activatable fibrinolytic inhibitor (TAFI), further protecting the formed fibrin plug.

In situations with impaired thrombin generation, e.g., hemophilia, a loose fibrin plug is formed, which is less resistant toward proteolysis. As a result, these patients are characterized by a defective sustained hemostasis and repeated rebleedings. A full thrombin burst is also dependent on the number of platelets resulting in the formation of loose fibrin plugs easily dissolved by fibrinolytic enzymes in patients with thrombocytopenia. This leads to the characteristic bleedings from tissues rich in fibrinolytic enzymes such as the gastric mucosa, the ear–nose–throat region, and the urinary tract seen in these patients.

Recombinant factor VIIa in hemophilia

Hemophilia patients have an intact TF pathway, which results in a normal platelet activation (bleeding time is normal in hemophilia). However, in hemophilic dogs, in which normal initial bleeding time was observed, frequent rebleedings and the formation of larger than normal hemostatic plugs rich in channels and areas of loosely packed platelets with an incomplete fibrin cap were observed [12]. Recently, the formation of loose fibrin clots with a high permeability constant, which was dose dependent with regard to FVIII/FIX, was reported in hemophilia plasma containing preactivated platelets following recalcification [13]. Furthermore, the impaired thrombin generation in hemophilia also may result in a suboptimal activation of FXIII and TAFI. Thus, the formation of defective hemostatic plugs that are sensitive to lysis and therefore fail to sustain hemostasis characterizes hemophilia.

FVII occurs in the normal circulation in a concentration corresponding to ~1% of the total FVII protein mass. Furthermore, purified FVIIa did not induce systemic activation of the coagulation system in a dog model used to identify potential thrombogenic factor in aPCC [2]. However, FVIIa as a hemostatic agent was a new concept in the 1970s. The vision at the time was to develop a therapy for hemophilia patients with inhibitors that would be as easily available and convenient as existing treatments for hemophilia patients without inhibitors. If the new agent were successful, there would be no further need for complicated, inconvenient, and expensive therapies such as induced immune-tolerance treatment. The first step in this development was the successful use of plasma-derived purified human FVIIa in two hemophilia patients with inhibitors without any signs of a general activation of the coagulation system [14]. To avoid the potential risk of transfer of pathogenic agents by plasma-derived products, recombinant technology was chosen for production of therapeutic FVIIa (rFVIIa) [15].

Following up on the early observations that pharmacologic doses of FVIIa may bind not only to TF but also to phospholipids, rFVIIa was found to bind weakly to thrombin-activated platelet in a cell-based *in vitro* model [11,16]. Through this TF-independent low-affinity binding, rFVIIa activates FX independent of the presence of FVIII or FIX and a dose-dependent increase in the thrombin generation on the preac-

tivated platelets occurs [2]. Although the lag phase of the initiation of thrombin generation normalized as compared with the value obtained in the presence of physiologic concentrations of clotting factors and platelets in the cell-based model, the height of the thrombin peak did not reach the same level as found in the physiologic situation after the addition of rFVIIa in concentrations of up to 500 nM (25–30 nM of FVIIa is the estimated plasma level following injection of the standard dose of 90 μg/kg, and 75–80 nM of FVIIa following the dose of 270 μg/kg). However, doses of 90 μg/kg or 270 μg/kg induce clinical hemostasis in most patients, indicating that the peak of thrombin generated may not be the most important, but rather the rate of thrombin generation. Furthermore, the clot lysis time *in vitro* in hemophilia plasma was found to be prolonged after the addition of rFVIIa [2]. Increased TAFI activation as a result of enhanced thrombin generation was suggested to contribute to the increased resistance to lysis [2]. However, a normalization of the fibrin permeability as a result of the tighter fibrin network demonstrated after addition of rFVIIa to hemophilia A plasma in the presence of preactivated platelets, should be of major importance for the increased resistance to proteolysis [13]. In the cell-based *in vitro* model, rFVIIa also was found to increase the rate and extent of platelet activation [2,4].

In summary, the hemostatic effect of rFVIIa in pharmacologic doses seems to be mediated by an enhanced rate of thrombin generation on thrombin-activated platelet surfaces. This will result in an increased further activation of platelets at the site of injury and increased platelet adhesion that may involve an enhanced platelet–platelet interaction initiated by thrombin binding to platelet glycoprotein Ib (GPIb) as well as other mechanisms. The enhanced thrombin generation ensures the formation of a tight fibrin structure of the hemostatic plug, as well as full activation of TAFI and FXIII (both activated by thrombin) necessary for maintaining hemostasis [2,4].

Clinical experience with recombinant factor VIIa in hemophilia patients with inhibitors

In the past, elective surgery has been more or less contraindicated in hemophilia patients with inhibitors because of the risk of uncontrollable bleeding. However, rFVIIa was demonstrated to have an efficacy rate of 90–100% in major surgery, including major orthopedic surgery [2,10]. Also, in patients with serious bleedings a similar efficacy rate was achieved using essentially the same dosing schedule as that recommended in surgery (90–120 μg/kg every 2 h during the first 24 h, thereafter the same dose with increasing intervals depending on type of surgery and clinical response) [2,4,10].

As part of the vision of providing a treatment for hemophilia patients with inhibitors that would make them similar to patients without inhibitors, the effect of rFVIIa in a home treatment setting was explored. An efficacy rate of 92% was achieved. However, the number of doses to achieve hemostasis was 2.2, which indicates that the dose used might not be optimal [2,15].

Dose adjustment and monitoring

By increasing the physiologic level of FVIIa, the nonspecific binding of rFVIIa to activated platelets is exploited. However, the exact relationship between the plasma concentration of FVII:C and the thrombin generation at the site of injury is not known. A number of assays for the measurement of thrombin generation have been described, but most of them measure thrombin formation in circulating blood rather than the thrombin generated at the site of injury. Various forms of thromboelastography as an assay of global hemostatic function have been suggested for monitoring rFVIIa treatment. Furthermore, it has been proposed as a tool for predicting optimal doses of rFVIIa in the treatment of hemophilia patients with inhibitors [17–19]. However, in two recently presented multicenter studies the inter- as well as intraindividual variations were found to be too large to allow any dose–response prediction in the clinical setting [20,21].

The mean recovery of rFVIIa (FVII:C at 10 min after injection) was found to be 46% (median 43%) [2]. The recommended dose (90–120 μg/kg) at 46% of recovery would then correspond approximately to 25–30 nM of FVIIa in plasma. However, clearance rate, recovery at 10 min after injection, and the capacity to generate thrombin on the platelet surface vary widely among individuals [2]. Accordingly, the optimal dose might show a great individual variation. Furthermore, the clearance rate in children below 15 years of age may be as much as three times the normal rate for adults [2], which suggests that they may require higher doses of rFVIIa in order to ensure formation of the firm, tight initial hemostatic plug that is necessary for maintaining hemostasis. Recently, a dose of 270 μg/kg was approved in Europe on the basis of studies comparing 90 μg/kg three times per bleed with one single bolus of 270 μg/kg [2].

Although treatment with rFVIIa is not a substitution therapy like FVIII/FIX therapy, the feasibility of administration of rFVIIa in a continuous infusion (CI) has been explored using various schedules [2]. The results include both successes and failures and may reflect the experience in hemophilia treatment at each center included, more than the dosing of rFVIIa. The initial experience of CI infusion of rFVIIa therapy pointed out the importance of the individual pharmacokinetics of each patient and recommended the dose schedule to be adjusted accordingly [22]. Extra bolus doses may be required in some patients to keep hemostasis, which requires extra attention from the medical staff. It is obvious, though, that rFVIIa may be administered as a CI, although the optimal dosing regimen is not known and may vary in different patients because of individual pharmacokinetics. Its success may also depend on the use of adjunct therapy like antifibrinolytics [2].

Effect of recombinant factor VIIa as prophylaxis

In noninhibitor patients with hemophilia, regular administration of FVIII/FIX several times weekly has been demonstrated to decrease the number of joint bleeds and thereby to minimize the development of arthropathy. Such treatment, therefore, is recommended in severe hemophilia patients [1,2]. In line with the initial vision of making the treatment of hemophilia patients with inhibitors similar to treatment of noninhibitor patients, it was considered important to investigate whether regular administration of rFVIIa to such patients would decrease the number of joint bleeds. The first report showing that administration of rFVIIa once daily prevented breakthrough bleeds during extensive physiotherapy following an orthopedic mechanical traction of a knee joint contracture was reported in 2000 by Cooper *et al.* at the World Federation of Hemophilia (WFH) Meeting in Montreal and published in 2001 [23].

Recently, several hemophilia patients with inhibitors have been successfully treated with repeated doses of rFVIIa, most of them having "target joints" characterized by an inflammatory synovitis and repeated bleeding episodes [2]. A randomized, prospective clinical trial using daily administration of rFVIIa in doses of 90 μg/kg or 270 μg/kg confirmed these results in finding decreased number of bleeds, not only during the 3-month treatment period but also during the observation time that followed (3 months of no regular treatment) [2]. This outcome may mark another step toward the goal of making the treatment of hemophilia patients with inhibitors similar to that of noninhibitor patients.

Amelioration of the inflammatory synovitis during the treatment period most probably contributed to the decreased number of bleeding episodes, although it is not clear how this effect was achieved by once-daily administration of an agent with a plasma half-life of 2–3 h. Neither is it clear why rFVIIa prophylaxis reduces the number of hemorrhagic events in the post-treatment period. Although the decrease in the inflammatory response may contribute to the decreased number of bleedings, evidence related to the extravascular distribution of FVIIa may also play a role in the prolonged effect of rFVIIa. TF–FVII complexes may form continuously on extravascular TF-expressing cells surrounding blood vessel walls [2,3]. The bound FVIIa is internalized and partially degraded in the cell, while some of it will reappear on the cell surface and bind to TF. This process may occur continuously until all FVII/FVIIa is cleared and may continue for a long time if there is plenty of FVIIa in the extravascular compartment [2], which may be the case after administration of pharmacologic doses of rFVIIa.

Assuming that a similar process occurs *in vivo*, continuous formation of rFVIIa–TF complexes on cell surfaces extravascularly may facilitate thrombin generation on platelets that plug the leak in blood vessels in the joint tissues following the mechanical strain of movement. A further support for the hypothesis that rFVIIa may be effective in prophylaxis in spite of no or marginal increase in the plasma level of FVII protein are the recently published results from continuous expression of canine FVIIa in hemophilic dogs [24]. In a hemophilia B dog receiving a low dose of a serotype 8 recombinant adeno-associated viral vector expressing canine FVIIa (AAV8–cFVIIa) gene transfer, no spontaneous bleedings were recorded during the 31 months of observation in spite of not achieving normalization of any of the clotting parameters (whole blood clotting time, thromboelastographic pattern) and no shortening of the PT. Furthermore, no cFVIIa antigen in plasma was demonstrated. Higher vector concentrations were shown to achieve sustained cFVIIa antigen concentration of 1.3–2.5 μg/ml plasma, which is similar to levels seen following a standard bolus dose of rFVIIa. In those hemophilia A dogs partially normalized clotting parameters were seen and there were no spontaneous bleeding episodes during the observation period of up to 18 months. No adverse effects were found. If applicable in humans, these observations indicate that continuous expression of rFVIIa may be useful in treatment of hemophilia patients with inhibitors. Furthermore, the results also indicate that the administration of rFVIIa in doses too low to appear in the plasma may induce hemostasis in the microcirculation and thereby prevent the development of joint bleeds. This may also support the hypothesis that extravascular rFVIIa may be of physiologic and pathologic importance [25].

Another reason for a prolonged functional activity of administered rFVIIa, also after it has disappeared from plasma, may be a binding to one or more proteins localized to the vessel wall and acting as reservoirs of rFVIIa. Thus, it was demonstrated that rFVIIa binds to endothelial cells with endothelial protein C receptor (EPCR) acting as the receptor [26]. Whether there are other binding sites to rFVIIa on endothelial cells is currently not clear.

Use of recombinant factor VIIa in other bleeding disorders

The ability of rFVIIa to enhance thrombin generation on the surface of activated platelets makes it a potential hemostatic agent in any situation that requires the formation of a tight hemostatic plug [2]. In the *in vitro* cell-based model, the addition of rFVIIa caused a dose-dependent shortening of the lag phase of platelet activation and thrombin generation on the activated platelets in the presence of platelet counts down to at least 10 000/μL. Also, a tighter fibrin structure was observed in the presence of rFVIIa and low platelet counts [2]. In the flow-chamber model the addition of rFVIIa to whole blood made thrombocytopenic increased the fibrin deposition [2]. If the events observed *in vitro* also occur *in vivo*, these may contribute to the hemostatic effect of rFVIIa in situations characterized by low platelet counts associated with uncontrolled hemorrhage [2]. Recently, evidence for an interaction between rFVIIa and the GPIbα may occur on activated platelets and thus may contribute to a hemostatic effect of rFVIIa

in patients with bleeding disorders [27]. Anecdotal reports of a hemostatic effect of rFVIIa in various platelet disorders, including various types of thrombocytopenia as well as functional platelet disorders such as Glanzmann thrombasthenia, Bernard–Soulier syndrome and others, have been reviewed recently [28].The use of rFVIIa in patients with Glanzmann thrombasthenia, who do not benefit from platelet transfusion, is approved by the European Medical Agency (EMEA). The dose recommended is similar to the hemophilia dosage: 70–120 μg/kg every other hour in serious bleeding and surgery [2]. A successful use of rFVIIa has also been reported in patients with von Willebrand disease type III, as well as in type II of the disease [2].

In patients with a normal basal hemostatic process, an impaired thrombin generation may occur when they are subjected to severe trauma or extended surgery. In these patients, a combination of dilution coagulopathy and local release of proteolytic enzymes caused by extensive tissue damage degrading coagulation proteins may develop. As a result of the dilution coagulopathy, loose, porous fibrin deposits may form, which will be easy targets for premature dissolution by released enzymes, leading to profuse, diffuse bleeding at sites of tissue damage. This process may be mainly localized without signs of generally increased fibrinolytic activity in the circulation. Successful use of rFVIIa in severely traumatized patients has been reported and rFVIIa has also been found effective in patients with uncontrollable hemorrhage unresponsive to conventional therapy [2]. A special situation characterized by profuse, massive bleeding is the postpartum bleedings. Successful use of rFVIIa, often administered as one single dose (90–100 μg/kg), has been reported in such patients. A review of 31 published but uncontrolled studies including 118 cases showed an efficacy rate of 90% of rFVIIa at a median dose of 71.6 μg/kg [29]. Furthermore, anecdotal reports of successful use of rFVIIa in cardiac and vascular surgery, as well as in uncontrollable postoperative bleedings, have been published [2]. Also, anecdotal reports of successful use of rFVIIa in patients with increased risk of bleeding as a result of treatment with anticoagulants are available [2].

Successful prophylactic use of rFVIIa in patients without any preformed coagulation disorder, but who were subjected to surgery expected to release an abundance of fibrinolytic enzymes such as surgical prostatectomy, has been reported. A single dose of rFVIIa administered immediately before the expected release of fibrinolytic enzymes may have helped to generate extra thrombin, resulting in the formation of tight fibrin plugs resistant to the fulminant fibrinolysis occurring locally. A single dose of rFVIIa was also reported to limit the growth of an intracerebral hematoma in patients with intracerebral hemorrhage [2].

An extensive report on 22 placebo-controlled, randomized trials using rFVII in nonhemophilia patients was recently published. The conclusion from this review was that the use of rFVIIa reduced the need for blood transfusion and may have reduced mortality. Furthermore, it did not increase the risk of venous thrombosis, but may have increased the risk of arterial thrombosis [2].

Safety

No side-effects have been observed in healthy volunteers [2]. During the time period from licensure to April 2003, 25 thromboembolic (TE) events from over 700 000 doses administered was reported in 2004, which was considered a remarkably low incidence of such events. The most recent safety update reports on adverse events (AE) from the approximately 800 000 standard doses of rFVIIa administered between 2003 and 2006. A total of 30 TE events and six TE-associated fatalities were found. It was concluded that despite the use of high-dose rFVIIa (270 μg/kg) in some clinical trials and registries, rFVIIa appears safe when used for congenital and acquired hemophilia. The prevalence of TE associated with rFVIIa use is less than 4 in 100 000 and a TE-associated fatality is also extremely rare. The report concludes, however, that use of rFVIIa for off-label indications should continue to be monitored closely [30].

No indication of the formation of inhibitory antibodies against rFVIIa was seen in patients with hemophilia or in nonhemophilia patients treated with rFVIIa. However, FVII-deficient patients are at risk for development of antibodies against FVII [2].

References

1 Hedner U. Prevention of arthropathy: May it be extrapolated from patients without inhibitors to patients with inhibitors? *Haemophilia* 2008; **14**(Suppl. 6): 23–7.

2 Hedner U. Factor VIIa and its potential therapeutic use in bleeding-associated pathologies. *Thromb Haemost* 2008; **100**: 557–62.

3 Rapaport SI, Rao LVM. The tissue factor pathway: How it has become a "prima ballerina." *Thromb Haemost* 1995; **74**: 7–17.

4 Hedner U, Ezban M. Tissue factor and factor VIIa as therapeutic targets in disorders of haemostasis. *Ann Rev Med* 2007; **59**: 29–41.

5 Niemetz J, Marcus AJ. The stimulatory effect of platelets and platelet membranes on the procoagulant activity of leukocytes. *J Clin Invest* 1974; **54**: 1437–43.

6 McGill M, Fugman DA, Vittorio N, *et al.* Platelet membrane vesicles reduced microvascular bleeding times in thrombocytopenic rabbits. *J Lab Clin Med* 1987; **109**: 127–33.

7 Lopez-Vilchez I, Escolar G, Diaz-Ricart M, *et al.* Tissue factor-enriched vesicles are taken up by platelets and induce platelet aggregation in the presence of factor VIIa. *Thromb Haemost* 2007; **97**: 202–11.

8 Kothari H, Rao LVM, Idell S, *et al.* Plasmin enhances cell surface tissue factor activity without increasing tissue factor antigen levels at the cell surfaces of mesothelial and endothelial cells. *Blood* 2007; **110**: Abstract 1758.

9 Bogdanov VY, Balasubramanian V, Hatchcock J, *et al.* Alternatively spliced human tissue factor: a circulating, soluble, thrombogenic protein. *Nature Med* 2003; **94**: 458–62.

10 Hedner U, Erhardtsen E. Potential role of rFVIIa as a hemostatic agent. *Clin Adv Hematol Oncol* 2003; **1**: 112–19.

11 Monroe D, Hoffman M, Roberts HR. Platelets and thrombin generation. *Arteriosler Thromb Vasc Biol* 2002; **22**: 1381–9.

12 Hovig T, Rowsell HC, Dodds WJ, *et al.* Experimental hemostasis in normal dogs and dogs with congenital disorders of blood coagulation. *Blood* 1967; **305**: 636–68.

13 He S, Blombäck M, Jacobsson Ekman G, *et al.* The role of recombinant factor VIIa (rFVIIa) in fibrin structure in the absence of FVIII/FIX. *J Thromb Haemost* 2003; **1**: 1215–19.

14 Hedner U, Kisiel W. Use of human factor VIIa in the treatment of two haemophilia A patients with high-titer inhibitors. *J Clin Invest* 1983; **71**: 1836–41.

15 Hedner U. Recombinant factor VIIa: Its background, development and clinical use. *Curr Opin Hematol* 2007; **14**: 225–9.

16 Monroe DM, Hoffman M, Oliver JA, *et al.* Platelet activity of high-dose factor VIIa is independent of tissue factor. *Br J Haematol* 1997; **99**: 542–7.

17 Sørensen B, Ingerslev J. Thromboelastography and recombinant factor VIIa—hemophilia and beyond. *Semin Hematol* 2004; **41**(Suppl. 1): 140–4.

18 Kawaguchi C, Takahashi Y, Hanesaka Y, *et al.* The in vitro analysis of the coagulation mechanism of activated factor VII using thromboelastogram. *Thromb Haemost* 2002; **88**: 768–72.

19 Young G, Blain R, Nakagawa P, *et al.* Individualization of bypassing agent treatment for haemophilia patients with inhibitors utilizing thrombelastography. *Haemophilia* 2006; **12**: 598–604.

20 Kenet G, Stenmo CB, Blemings A, *et al.* Intra-subject variability of thromboelastographic parameters following rFVIIa in vivo administration in haemophilia patients: A randomized multicentre trial. *J Thromb Haemost* 2007; **5**(Suppl. 2): Abstract.

21 Young G, Ebbesen LS, Viuff D, *et al.* Evaluation of thromboelastography for monitoring recombinant activated factor VII ex vivo in haemophilia A and B patients with inhibitors: A multicentre trial. *Blood Coagul Fibrinolysis* 2008; **19**: 276–82.

22 Schulman S, Bech Jensen M, *et al.* Feasibility of using recombinant factor VIIa in continuous infusion. *Thromb Haemost* 1996; **75**: 432–6.

23 Cooper HA, Jones CP, Campion E, *et al.* Rationale for the use of high dose rFVIIa in a high-titre inhibitor patient with haemophilia B during major orthopaedic procedures. *Haemophilia* 2001; **7**: 517–22.

24 Margaritis P, Roy E, Aljamali MN, *et al.* Successful treatment of canine hemophilia by continuous expression of canine FVIIa. *Blood* 2009; **113**: 3682–9.

25 Hedner U. Potential role of recombinant factor FVIIa in prophylaxis in severe hemophilia patients with inhibitors. *J Thromb Haemost* 2006; **4**: 2498–500.

26 Ghosh S, Pendurthi UR, Steinoe A, *et al.* Endothelial cell protein C receptor acts as a cellular receptor for factor VIIa on endothelium. *J Biol Chem* 2007; **282**: 11849–57.

27 Weeterings C, de Groot PG, Adelmeijer J, *et al.* The glycoprotein Ib-IX-V complex contributes to tissue factor-independent thrombin generation by recombinant factor VIIa on the activated platelet surface. *Blood* 2008; **112**: 3227–33.

28 Hers I, Mumford A. Understanding the therapeutic action of recombinant factor VIIa in platelet disorders. *Platelets* 2008; **19**: 571–81.

29 Franchini M, Franchi M, Bergamini V, *et al.* A critical review on the use of recombinant factor VIIa in life-threatening obstetric postpartum hemorrhage. *Semin Thromb Hemost* 2008; **34**: 104–12.

30 Abshire T, Kenet G. Safety update on the use of recombinant factor VIIa and the treatment of congenital and acquired deficiency of factor VIII or IX with inhibitors. *Haemophilia* 2008; **14**: 898–902.

25 Products used to treat hemophilia: dosing

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The temporary correction of the coagulation defect is the mainstay of treatment in hemophilia. However, the "ideal" dose of factor VIII (FVIII) or FIX that needs to be administered to invariably achieve hemostasis without "overtreating" is unknown. To date, there are no controlled clinical trials that address this important question, mainly because of the ethical implications of reducing dosing to a threshold of bleeding symptoms. Dosing for hemophilia treatment has been arrived at by empirical assessment, essentially "trial and error" based on the pharmacokinetics of the factors and the characteristics of replacement product [1].

Historical background

Because hemarthroses constitute the most common manifestation of severe hemophilia, the prevention of this morbid clinical state has been long studied. Brinkhous and collaborators treated hemophilic dogs in the initial studies in 1947 and continued to study outcomes of therapy for more than 20 years. When plasma was given every 3.5 days to the animals, the frequency and severity of hemarthrosis decreased until there was no evidence of joint disease when compared with the administration of small and infrequent transfusions of plasma [2,3]. These observations were fundamental for the understanding of dosing in prophylaxis.

In the 1950s, Brinkhous and his group [4], and Biggs and Macfarlane [5], working independently, reported their observations in the treatment of bleeding in patients with hemophilia A. Their conclusion was that to maintain adequate hemostasis in these individuals, FVIII levels of at least 35% of normal are necessary for treating minor injuries and levels of around 50% of normal are required for major trauma or surgery.

In the 1960s, cryoprecipitate and a fraction of human plasma (fraction I of Cohn) became available as alternative sources of therapy for patients with hemophilia A. One of the advantages of these products was the need for volume when compared with plasma [6,7]. Subsequently, several FVIII and FIX concentrates became available for the treatment of hemophilia A and B, respectively, significantly facilitating the management of these conditions.

Based on the principle that moderate hemophiliacs (FVIII or FIX level above 1%) rarely develop severe disabling arthropathy [8–10], multiple studies were performed between 1967 and 1982 using low doses of factor VIII and IX for the treatment of acute hemarthrosis with variable results (Table 25.1).

In most cases, treatment was administered within the first few hours of onset of bleeding. It is interesting to note that even very low doses of factor replacement (less than 10 U/kg) were effective in 73–100% of cases. Possible explanations for some of these results are: (i) Studies were carried out during summer camps for hemophilic patients during which close surveillance and early treatment were the norm; (ii) infusions were administered for traumatic hemarthroses only; (iii) target joints were not included; and (iv) only mild and moderate bleeds were assessed. Further studies by Aronstam *et al.* [11,12] evaluated different treatment regimens versus severity of the hemarthroses. As expected, lower doses of factor replacement were associated with higher failure rates in target joints and bleeds that were ongoing for some time prior to administration of replacement therapy.

Pharmacokinetics and dosage calculations

The appropriate dose of FVIII or FIX for replacement therapy is an amount of the relevant clotting factor that will provide satisfactory hemostasis to control a bleeding episode [13]. Regardless of the product used, the dose of FVIII or FIX should be calculated in terms of units per kilogram of body weight. All calculations should be made with the assumption that one unit of FVIII per kilogram of body weight will raise the circulating FVIII level by about 0.02 U/mL, and one unit of FIX per kilogram of body weight will raise the plasma FIX by 0.01 U/mL. Much of the difference in expected recovery between FVIII and FIX is a result of variable volumes of distribution. FVIII circulates almost exclusively intravascularly and FIX diffuses into the extracellular water space [14]. Thus, to correct for interpatient variability after infusion of FVIII

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Table 25.1 Treatment of hemarthrosis with low doses of factor VIII and IX.

Dose (U/kg body weight)	Factor plasma level (%)	Number of treated episodes	Success rate (%)	Therapeutic material	Type of bleed	Reference
23	24–33	25	56–64	Cryo	Hemarthrosis	27
20–30	40–50	51	92	FVIII, other	Hemarthrosis	26
10		51	96	FVIII	Hemarthrosis	13
7–9		106	90	FVIII	Hemarthrosis	28
11–13		173	79			
15–17		64	94			
8–12		62	100	FVIII	Hemarthrosis, other	29
7.5–12.5	15–25	196	89	FVIII	Hemarthrosis, other	30
12.5–20	25–40	349	94			
3–7		60	100	FVIII/FIX	Hemarthrosis	31
31	53	144	99	Cryo	Hemarthrosis, other	32
7		119	73	FVIII, other	Hemarthrosis	33
14		134	75			
28		86	64			
11–16		144	78	FVIII, other	Hemarthrosis	34
7		95	89	FVIII	Hemarthrosis	35
14		106	77			

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and FIX products, individual pharmacokinetic studies can be performed to optimize dosing.

The dose needed to achieve hemostasis varies widely and choice of dose needs to be calculated taking into account a number of parameters: severity of the bleeding episode; pharmacologic properties of the clotting factors, which include the half-disappearance time; and the *in vivo* recovery based on the volume of distribution within the vascular compartments. The doses suggested in Table 25.2 serve as a guide to calculate the approximate amount required and are not based on randomized clinical trials. Therapeutic infusion of replacement factor should be administered as early as possible in an attempt to prevent permanent damage to joints and soft tissues and should continue until adequate hemostasis has been achieved or wound healing is complete. Bleeding complications in association with surgical procedures can be seen in 4–23% of cases, usually in the postoperative period rather than during the surgery [15–18].

Factor replacement can be administered by either intermittent bolus or continuous infusion. Some advantages of the latter are the following: total factor use may decrease by as much as 30% [19,20]; achievement of a faster steady state in plasma; maintenance of a constant therapeutic factor level; and avoidance of peaks and troughs, which facilitates laboratory monitoring [21] (for further details on continuous infusion see Chapter 7). Of note, when continuous infusion is started, the dose needed is often higher during the first few postoperative days because of the rapid clearance of the factor in the immediate postoperative period [22].

Ideally, for individuals who must undergo elective major surgical procedures, an *in vivo* recovery and half-life study should be performed during a nonbleeding state with a 3–5 half-life wash-out period. A dose of the therapeutic product to increase the plasma level to 100% should be used and samples for factor activity should be drawn at times 0, 30 min, 60 min, 3, 6, 9, 24, 28, 32 and 48 h post infusion. For FIX, additional time points at 50 and 72 h should be drawn given the longer half-life [23].

Based on the pharmacokinetic study of the individual, an initial "loading" dose and subsequent doses can be calculated. For example, in an individual with FVIII deficiency and a normal recovery and half-life, factor dosing can be calculated using the following formula:

Table 25.2 Guidelines for factor replacement in severe and moderate hemophilia A and B.

Site of hemorrhage	Optimal factor level (%)	Dose (U/kg body weight)		
		Factor VIII	Factor IX	Duration in days
Joint	30–50	15–25	30–50	1–2
Muscle	30–50	15–25	30–50	1–2
Gastrointestinal tract	40–60	30–40	40–60	7–10
Oral mucosa	30–50	15–25	30–50	Until healing
Epistaxis	30–50	15–25	30–50	Until healing
Hematuria	30–50	15–25	30–50	Until healing
Central nervous system	80–100	50	80–100	10–21
Retroperitoneal	50–100	30–50	60–100	7–14
Trauma or surgery	50–100	30–50	60–100	Until healing

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Initial loading dose = (desired FVIII level – patient baseline FVIII) × body weight (kg) × 0.5 unit/kg

Assuming that 8–12 h after the initial bolus the plasma level will decrease by about 50%, further doses can be given of one-half of the loading dose every 8–12 h. If constant infusion is employed, the initial loading dose should be divided by 12, which is equal to the number of units per hour of FVIII concentrate. For example, to raise the FVIII level to 100% in a 70-kg individual with less than 1% activity, the initial loading dose will be 3500 units followed by boluses of 1750 units every 8–12 h. For continuous infusion, 292 units per hour will be the calculated dose in this scenario. FVIII levels should be monitored and ongoing dosing adjusted accordingly.

The dose calculations for FIX concentrates are different from those used in FVIII deficiency because the recovery of infused FIX is lower (~50%) owing to the diffusion over a larger volume. In addition, there is some evidence to suggest that FIX binds to elements on the vessel wall, more specifically to collagen type IV [24]. Thus, to raise to 100% of normal a 70-kg severely affected patient, 7000 units should be given as a bolus, followed by half this amount every 12–18 h. The continuous infusion dosing for this scenario can be calculated by dividing the loading dose by 24, i.e., approximately 292 units of FIX per hour [25].

For those individuals with a rapid initial phase decay or consumption (more than 50% decline in 6 h), a second bolus of FVIII or FIX can be given (approximately 50% of initial bolus) within 3–6 h of starting the surgery to avoid excessive intraoperative and immediate postoperative hemorrhage. Thereafter, half or more of the initial loading dose should be readministered every 12 h in order to maintain nadir factor levels greater than 50%. Factor activity should be measured daily and adjusted to maintain the desired level (see Table 25.2).

In the 1970s, home therapy was introduced as supplies of factor production became more widely available and as self-infusion was taught more commonly. This made a great impact on the treatment of hemophilia. This mode of treatment has substantially improved the quality of life of these individuals, especially those with severe hemophilia, as it reduces visits to the hospital and prevents long-term complications, such as arthropathy, when early treatment is initiated. As an indication for home infusion, a minimal spontaneous bleeding episode is defined as any symptom of pain or distress recognized by the patient in a joint or soft-tissue space. Minimal bleeding or hemarthrosis at an early stage may not be associated with significant edema, erythema, or heat, and usually there is no known trauma [13]. Doses as small as 10 U/kg of FVIII or FIX have been proven to be effective in this type of bleeding and theoretically will lead to a plasma level of approximately 20% in FVIII or 10% in FIX activity. Such dosing proved successful in the management of 49 out of 51 early joint hemorrhages in hemophilia A patients described by Abildgaard in 1975 [13]. It should be noted that this dose is not adequate for full-blown hemarthroses or for bleeding in critical anatomical areas (head and neck, throat, wrists, hand, foot, abdomen, or gastrointestinal tract).

Moderate bleeding episodes (hemarthrosis, advanced soft-tissue hemorrhage) often respond to an early infusion of 20–25 U/kg, which will correspond to a plasma level of 40–50% FVIII activity or 20–25% FIX activity. Honig *et al.* [26] reported successful treatment of acute hemarthrosis in 48 of 51 episodes using a single dose of 20–30 U of FVIII per kilogram of body weight. In many of these individuals, a single infusion is sufficient to control the bleeding; however, if no improvement is noted in 12–24 h or if significant symptoms persist, a second infusion should be administered.

During severe bleeding episodes (central nervous system, surgical procedures, and severe trauma) larger doses of replacement therapy are necessary. In addition, maintenance doses are needed to sustain hemostatic levels until bleeding is controlled or, if surgery is required, until the wound is well healed. This may take up to 10–20 days of replacement therapy depending on the surgery. For FVIII-deficient individuals, an initial infusion of 40–50 U/kg should be sufficient to obtain hemostasis in this context and levels should be maintained by repeated doses of at least 20–25 U/kg at approximately 12-h intervals to maintain physiologic circulating levels of the deficient clotting factor for a specified duration (see Table 25.2). Factor IX replacement should be started with an initial bolus of 80–100 U/kg followed by repeated doses of 40–50 U/kg every 18–24 h.

Treatment guidelines for specific bleeding episodes

Mouth and neck region

Bleeding from the floor of the mouth, pharynx, or epiglottic area can result in partial or complete airway obstruction. External compression of airway because of hemorrhage can also be seen after placement of neck or subclavian catheters in hemophilic individuals. Hence, such bleeding should be treated with aggressive replacement therapy of the deficient factor until complete resolution of the bleeding is established. Doses to maintain factor levels above 80% should be the goal of treatment.

Complicated joint bleeds

Hip joint or acetabular hemorrhages are of major concern because of increased intra-articular pressure from accumulated blood. The concomitant inflammation may lead to aseptic necrosis of the femoral head. Replacement therapy should start promptly. These individuals can be treated with twice-daily infusions to sustain a factor level above 30% for at least 3 days, along with enforced bed rest.

Iliopsoas hemorrhages

Iliopsoas bleeds are less frequently encountered in young children. Together with pain, common clinical manifestations of iliopsoas bleeding are upward flexion of the thigh, discomfort on passive extension, and decreased sensation over the ipsilateral thigh owing to compression of the sacral plexus root of the femoral nerve. Twice-daily infusion should be administered to maintain a factor level above 20% for about 3 days followed with daily infusions until symptoms decrease. Bed rest should be enforced.

Compartment syndrome

Bleeding into closed-compartment muscle and tissue areas, such as hand, wrist, forearm, and anterior or posterior tibial compartments, may result in compression of nerves and blood vessels. Initial symptoms such as pain and edema can be preceded by paresthesias and loss of distal pulses. Prompt treatment with replacement factor is indicated to maintain levels of around 50–100% of normal. If replacement therapy fails to stop the progression, surgical decompression may be indicated.

Central nervous system hemorrhages

These are usually traumatic in origin and should be considered an emergency until proven otherwise by imaging studies. Factor infusion should be given immediately, even prior to imaging studies and neurologic consultation. FVIII or FIX levels should be kept at 80–100% of normal. Late bleeding after head trauma can manifest as long as 3–4 weeks after the injury. Hence, patients with head trauma should be infused immediately unless the injury is proven insignificant.

Following treatment of the acute episode, which usually is of approximately 2 weeks duration, prophylactic treatment for about 6 months is usually indicated to decrease the possibility of a recurrent intracerebral hemorrhage. Doses of about 40 U/kg of FVIII every other day and 50 U/kg of FIX twice weekly should be given [22].

Hematuria

Gross spontaneous and asymptomatic hematuria is not uncommon in the hemophilia population. Trauma, calculi, and infections should be ruled out, and treatment with increased oral or intravenous fluids, bed rest, and a short course of corticosteroids (i.e., prednisone 0.5/kg/day) for 3–4 days is usually sufficient to arrest the bleeding. If symptoms persist then therapy with the deficient factor should be given at a dose to keep plasma levels of about 30–50% of normal until complete resolution of the hematuria. Antifibrinolytic agents, such as tranexamic acid or ε-aminocaproic acid, are contraindicated in individuals with hematuria because of the risk of forming clots in the urinary tract and producing obstruction.

Other

For certain particular circumstances, such as aggressive rehabilitation after orthopedic surgery, prophylactic replacement therapy is indicated. Doses of 20–30 U/kg of FVIII or 40–60 U/kg of FIX on the day of therapy should be sufficient to prevent hemorrhages.

References

1 Escobar MA. Treatment on demand—in vivo dose finding studies. *Haemophilia* 2003; **9**: 360–7.
2 Swanton MC. Hemophilic arthropathy in dogs. *Lab Invest* 1959; **8**: 1269.
3 Brinkhous KM, Swanton MC, Webster WP, Roberts HR. In: Vanderfield IR (ed.) *Dosing. Hemophilic arthropathy transfusion therapy in its amelioration—canine and human studies*. Sydney, Australia: The Haemophilia Society of NSW, 1966: 18–21.
4 Brinkhous KM, Langdell RD, Penick G, *et al.* Newer approaches to the study of haemophilia and haemophilioid states. *JAMA* 1954; **154**: 481.
5 Biggs R, Macfarlane RG. Haemophilia, Christmas disease and related conditions. In: Biggs R, Macfarlane RG (eds.) *Human Blood Coagulation and its Disorders*, 2nd edn. Springfield, IL: Charles C Thomas, 1957: 239–74.
6 Pool JG, Hershgold EJ, Pappenhagen AR. High-potency antihaemophilic factor concentrate prepared from cryoglobulin precipitate. *Nature (London)* 1964; **203**: 312–13.
7 McMillan CW, Diamond LK, Surgenor DM. Treatment of classic hemophilia: The use of fibrinogen rich in factor VIII for hemorrhage and for surgery. *N Engl J Med* 1961; **265**: 277.
8 Ramgren O. Haemophilia in Sweden, III. Symptomatology, with special reference to differences between haemophilia A and B. *Acta Med Scand* 1962; **171**: 237.
9 Biggs R, Macfarlane RG. Haemophilia and related conditions: A survey of 187 cases. *Br J Haematol* 1958; **4**: 1.
10 Kasper CK, Dietrich, SL, Rapaport SI. *Hemophilia Prophylaxis with AHF Concentrate*. XII Congress International Society of Hematology. New York; 1968: 176.
11 Aronstam A, Wassef M, Hamad Z, *et al.* A double-blind controlled trial of two dose levels of factor VIII in the treatment of high risk haemarthroses in haemophilia A. *Clin Lab Haematol* 1983; **5**: 157–63.
12 Aronstam A. Prevention of haemophilic arthropathy. *Folia Haematol* 1990; **117**: 499–504.
13 Abildgaard CF. Current concepts in the management of hemophilia. *Semin Hematol* 1975; **12**: 223–32.
14 Berntorp E, Bjorkman S. The pharmacokinetics of clotting factor therapy. *Haemophilia* 2003; **9**: 353–9.
15 Kasper CK, Boylen AL, Ewing NP, *et al.* Hematologic management of hemophilia A for surgery. *JAMA* 1985; **253**: 1279–83.
16 Lachiewicz PF, Inglis AE, Insall JN, *et al.* Total knee arthroplasty in hemophilia. *J Bone Joint Surg Am* 1985; **67**: 1361–6.
17 Kitchens CS. Surgery in hemophilia and related disorders. A prospective study of 100 consecutive procedures. *Medicine (Baltimore)* 1986; **65**: 34–45.
18 Rudowski WJ, Scharf R, Ziemski JM. Is major surgery in hemophiliac patients safe? *World J Surg* 1987; **11**: 378–86.
19 Hathaway WE, Christian MJ, Clarke SL, Hasiba U. Comparison of continuous and intermittent Factor VIII concentrate therapy in hemophilia A. *Am J Hematol* 1984; **17**: 85–8.

20 Rochat C, McFadyen ML, Schwyzer R, *et al.* Continuous infusion of intermediate-purity factor VIII in haemophilia A patients undergoing elective surgery. *Haemophilia* 1999; **5**: 181–6.
21 Goldsmith JC. Rationale and indications for continuous infusion of antihemophilic factor (factor VIII). *Blood Coagul Fibrinolysis* 1996; **7**(Suppl. 1): S3–6.
22 Lusher J. Hemophilia A and B. In: Lilleyman JS, Hann IM, Blanchette VS (eds.) *Pediatric Hematology*, 2nd edn. London: Churchill Livingstone, 1999: 585–600.
23 Lee M, Morfini M, Schulman S, Ingerslev J, and the Factor VIII/Factor IX Scientific and Standardization Committee of the International Society for Thrombosis and Haemostasis. Scientific and Standardization Committee Communication. The design and analysis of pharmacokinetic studies of coagulation factors. International Society on Thrombosis and Haemostasis. Available at: http://67.199.103.212/Publications/OfficialCommunications/Factor8and9/Pharmacokinetic/tabid/149/Default.aspx (accessed on 3 December 2009).
24 Wolberg A, Stafford, DW, Erie DA. Human factor IX binds to specific sites on the collagenous domain of collagen IV. *J Biol Chem* 1997; **272**: 16717–20.
25 Roberts HR, Escobar MA. Other coagulation factor deficiencies. In: Loscalzo J, Schafer AI (eds.) *Thrombosis and Hemorrhage*, 3rd edn. Philadelphia: Lippincott Williams & Wilkins, 2003: 575–98.
26 Honig GR, Forman EN, Johnston CA, *et al.* Administration of single doses of AHF (factor VIII) concentrates in the treatment of hemophilic hemarthroses. *Pediatrics* 1969; **43**: 26–33.
27 Brown DL, Hardisty RM, Kosoy MH, Bracken C. Antihaemophilic globulin: Preparation by an improved cryoprecipitation method and clinical use. *BMJ* 1967; **2**: 79–85.
28 Penner JA, Kelly PE. Lower doses of factor VIII for hemophilia. *N Engl J Med* 1977; **297**: 401.
29 Ashenhurst JB, Langehannig PL, Seeler RA. Early treatment of bleeding episodes with 10 U/kg of factor VIII. *Blood* 1977; **50**: 181–2.
30 Weiss AE. Doses of Factor VIII for hemophilic bleeding. *N Engl J Med* 1977; **297**: 1237–8.
31 Ripa T, Scaraggi FA, Ciavarella N. Early treatment of hemophilia with minimal doses of factor VIII or factor IX. *Blood* 1978; **51**: 763.
32 Allain JP. Dose requirement for replacement therapy in hemophilia A. *Thromb Haemost* 1979; **42**: 825–31.
33 Aronstam A, Wasssef M, Choudhury DP, *et al.* Double-blind controlled trial of three dosage regimens in treatment of haemarthroses in haemophilia A. *Lancet* 1980; **i**: 169–71.
34 Aronstam A, Wassef M, Hamad Z, Aston DL. The identification of high-risk elbow hemorrhages in adolescents with severe hemophilia A. *J Pediatr* 1981; **98**: 776–8.
35 Aronstam A, Wassef M, Hamad Z. Low doses of factor VIII for selected ankle bleeds in severe haemophilia A. *BMJ* 1982; **284**: 790.

26 Products used to treat hemophilia: regulation

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Introduction

Hemophilia care consists of many components [1]. The provision of concentrates of the deficient coagulation factors is an essential component and their safety, quality, and efficacy need to be assured independently of the measures dictated by the market and the individual manufacturers. Over the past 20 years, this assurance has become the role of regulatory authorities. Compared with other products of pharmaceutical manufacture, the regulation of hemophilia products is a relatively recent phenomenon. The products of industrial-scale plasma fractionation have been subject to the oversight of the United States Food and Drugs Administration (FDA) since the 1940s, because of the products' status as biologics, subject to a regulatory framework that is over a century old. In Europe, the evolution of a system of harmonized and centralized approval of medicinal products in the European Union initially exempted plasma derivatives [2] and only began to incorporate them in 1989 [3]. The factors contributing to the significant heightening of this supervision over the past 20 years have been reviewed [4].

These factors have resulted in a regulatory framework in the developed world that assesses hemophilia products as medicines in the highest category of risk relative to other therapeutic agents. It is noteworthy that systems of official regulation mandating standards and other measures are now coupled with voluntary standards adopted by industry bodies as additional features of a comprehensive nexus of arrangements contributing to product quality and risk minimization [5]. While the requirements now in place in the European Union demonstrate a comprehensive range of measures in that market (Figure 26.1), they are fairly representative of the systems in place worldwide in terms of the aspects of product manufacture that they address, and the target outcomes. The principles underlying these product-related measures have been discussed [6], and while the principal focus has been on the products of industrial plasma fractionation, the lessons learned through these products are now reflected in the requirements for the products of recombinant technology.

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Underpinning these measures is the unspoken but practiced concept of "zero risk" in blood product manufacture and delivery. While this is a natural outcome of past failures, it has led to a regulatory framework that appears to be detached from the standard risk management and cost-effective, evidence-based principles that shape modern healthcare delivery. While product safety is paramount, some of the measures introduced and embedded in current practice are difficult to quantitate in terms of safety, while their effect on supply and delivery can be profound.

Products of local and blood bank production

The option of delivering product from local production in mainstream blood banking environments generally exists only for hemophilia A through the production of cryoprecipitate (cryo). Cryo in blood banks may be produced in a closed system of blood bags and then lyophilized to increase its convenience [7]. The following features of the product need to be kept in mind:

- cryo is a crude product that will not meet criteria for high-purity plasma concentrates, such as potency, purity, and solubility. However, this is not a significant problem in terms of its safety;
- the ability to characterize the product through representative batch sampling is limited. In other words, it is not possible to label a vial of freeze-dried cryo for potency;
- viral reduction techniques are not easily applied to the manufacture of cryo. This is because they are based on technology not easily adapted to blood centers, and because the low purity of the product prevents inactivation through heat.

The issues underpinning access to cryo have been reviewed [8]. In particular, the safety aspects have been emphasized by Evatt *et al.* [9], who have shown that, in the absence of additional safety measures, the risk of HIV infection in individuals with hemophilia exposed to cryo over a lifetime is significant (Table 26.1). Enhancement of cryo safety could include the selection of donors from low-risk populations, whose plasma could be quarantined until the donor has been recalled and retested for markers of infection. All such testing should be done with the most sensitive tests possible and using intervals

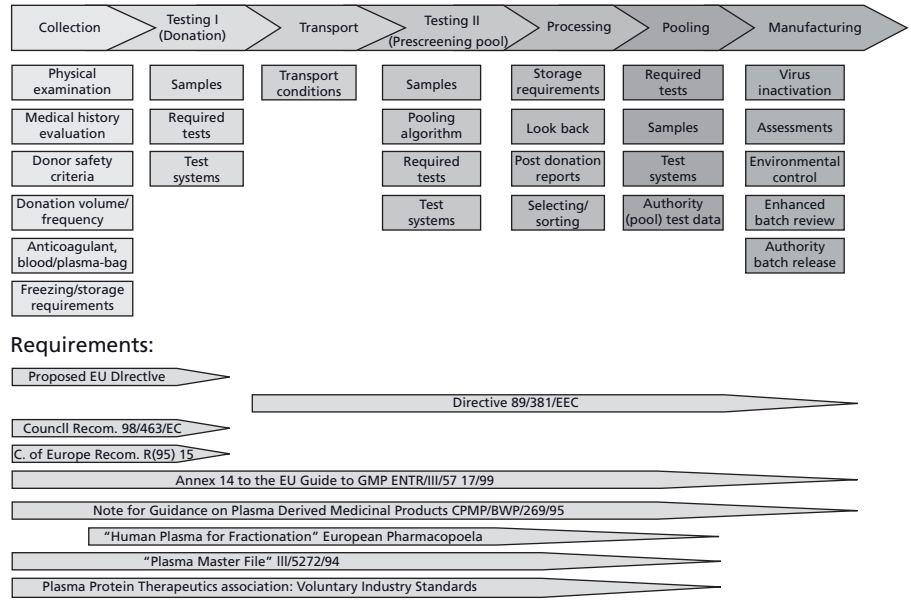

Figure 26.1 Systems of oversight for assuring product quality and safety in the plasma products sector in Europe (after J. Bult, unpublished observations).

Table 26.1 Risk (%) that a person with hemophilia in Venezuela or the USA will be exposed to HIV-contaminated blood products, based on years of treatment and risk of an HIV-infected donation.

Venezuela	USA			
Years of treatment	Lower (1/25 700)[a]	Mid (1/21 200)[a]	Upper (1/17 500)[a]	Mid (1/545 100)[a]
5	3.4	4.2	5.0	0.16
10	6.8	5.1	9.8	0.33
15	10.0	12.0	14.3	0.49
20	13.1	15.6	18.6	0.66
30	19.0	22.5	26.6	0.99
40	24.4	28.8	33.7	1.3
50	29.5	34.6	40.2	1.6
60	34.3	39.9	46.0	2.0

[a]Estimated risk for HIV-infected donation.
Reproduced from Evatt *et al.* [9] with permission.

that allow donors to seroconvert for the transfusion-transmitted viruses in case they were infected at the time of donation. Using nucleic acid testing (NAT), the viral window period for the important viruses can be substantially decreased. Pools of dedicated plasma donors, carefully selected and repeatedly tested, can, in time, become a source of very safe raw material, compared with first-time donors.

Some approaches to the viral inactivation of cryo have been described [10]. Any level of viral inactivation will result in a drop in factor VIII (FVIII) yield, and therefore the optimization of yields using improved plasma handling techniques is necessary if the safety and supply of cryo are to be enhanced. The pharmacologic stimulation of donors to produce more FVIII may be worth considering as a means of improving yields [11].

A recently described method uses solvent/detergent technology, established as the benchmark method for inactivation of the highly pathogenic enveloped blood-borne viruses, to produce virally inactivated cryo in a closed-bag blood system [12]. This method holds great promise for the delivery of safe cryo in emerging countries.

The nature of the manufacturing process for cryo results in a limitation in the ability to exert many of the standard features of pharmaceutical quality control that are possible for concentrates. In particular, the absence of sizeable batches makes homogeneous batch sampling, characterization, and labeling of ingredients of interest (active and impurities) difficult. Nevertheless, the ability to impose quality system management and adherence to good manufacturing practices is nowadays considered to be universally applicable. There are several standards and regulations in the international regulatory environment, which are applied for the manufacture and characterization of cryo. The US Code of Federal Regulations (CFR) [13] includes requirements for cryo that ensure that the measures underpinning whole-blood transfusion safety in the USA will be reflected in cryo. These measures include NAT, which will enhance cryo safety as discussed above. In Europe, centralized oversight of blood components including cryo is now in place with the recent introduction of the Blood Directive of the European Commission [14], which has replaced the current national requirements for these products. This directive is underpinned by technical standards for safety and quality. Standards such as those recommended by the Council of Europe [15] are being introduced. Such standards currently do not include NAT, which is not in universal use in the European environment at the blood donor level. Therefore, alignment to the evolving European regulatory

framework will not, as currently perceived by this author, enhance the safety of cryo to the level found in the USA.

In summary, cryo may be subjected to a level of regulatory oversight that, while unable to assure safety and quality to the standards available for concentrates, can result in these properties being reflective of the safety of the local blood supply. Any additional measures, such as the introduction of viral inactivation for cryo, cannot be supported by the regulatory requirements for these measures for concentrates, as these demand substantial validation and extensive studies, unavailable in blood centers. Therefore, while much can be done to improve the safety of cryo as a therapeutic modality in the developing world, it can never substitute for concentrates in terms of safety and quality that can be assured by regulatory oversight.

Products of large-scale plasma fractionation

Plasma concentrates are similar to conventional pharmaceuticals in that they are produced in large batches from a homogeneous pool of starting material, through well-defined processes subject to the tenets of standard pharmaceutical quality control. However, biologic drugs such as factor concentrates cannot be considered as generic agents, and each manufacturing process requires individual assessment with full product specification. While general properties leading to quality and safety may be reflected in standards of the pharmacopeia, the range of approaches to the manufacture of FVIII and factor IX (FIX) concentrates developed over the past 40 years has resulted in significant differences between products, which require thorough evaluation for their potential effect on the factors of interest and the impurities in the products.

Regulatory agencies oversee the introduction and maintenance of factor concentrates on the market through a set of well-defined principles that are common across the developed world. These include (i) facility licensure (good manufacturing practice; GMP); (ii) premarket product assessment; and (iii) postmarket surveillance.

Facility licensure

Licensure of plasma fractionation plants is done through reference to codes of GMP, which are generally generic documents specifying quality standards for manufacture that are common to all medicinal products. GMP seeks to ensure that manufacture is consistently carried out to high standards such that product safety, quality, and consistency are assured. Following inspections, which may identify deficiencies, the regulator and the manufacturer generally collaborate to ensure the issue of a manufacturing license, which will allow production to a high standard. Recently, the Pharmaceutical Inspectorate Convention has adopted a GMP for medicinal products that includes a chapter specifically addressing plasma-derived products [16]. The requirements of this chapter are shown in Table 26.2.

Table 26.2 Principles of good manufacturing practice for plasma fractionation agencies (Pharmaceutical Inspectors Convention Scheme 2003).

Quality management
Premises and equipment
Blood and plasma collection
Traceability and post collection measures
Production and quality control
Retention of samples
Disposal of rejected blood, plasma, or intermediates

The importance of GMP in assuring product safety is recognized by the regulator and product manufacturer alike. The ability of the latter to manufacture product consistently to a similar, predefined, and high manufacturing standard is pivotal to safety and depends on GMP. Examples of breakdowns in GMP impacting on product safety are nowadays rare owing to the high standards of manufacture evolved over the past 20 years, but such incidents have been implicated in product safety problems including viral transmission because of inadequate segregation between previral and postviral inactivation streams [17]. With viral inactivation processes, it is impossible to subject product batches to final testing to assure adequate viral elimination, and reliance on GMP for this is absolute. The whole of the manufacturing chain requires adherence to GMP, and its presence in collection and testing procedures is strictly enforced by regulators [18]. Voluntary industry standards have been introduced for ensuring that the minimal measures enforced by regulators are buttressed by additional measures for areas such as the processing of fractionation intermediates from different sources [19].

Premarket product approval

The assessment of the manufacturing process and the features of the product are done through the submission of product dossiers, which describe these in great detail. The main agencies—the FDA and the EMEA—have standard formats for the submission of data for manufacturing, preclinical, and clinical assessment. The data for the description and validation of the viral inactivation steps incorporated into the manufacture are a crucial component of these submissions. The EMEA has issued detailed guidance for the performance of such studies [20]. Such guidance is a feature of the regulatory framework of all the major agencies, and provides the industry with state-of-the-art assessment and regulation of the aspects related to the safety, quality, and efficacy of plasma derivatives.

International efforts for the harmonization of regulatory procedures have included the development of standardized approaches for the collation and presentation of data for

regulatory review, through the so-called Common Technical Document (CTD). The International Conference for Harmonization (ICH) has developed CTDs for the assessment of several aspects of safety, quality, and efficacy [21]. These documents provide a structured path for manufacturers to compile data in a form that is easily assessable, and their use for plasma derivatives, while still at an early stage, should contribute to further streamlining of the regulatory process.

An essential component of the premarket approval process is the demonstration of product pharmacokinetics and efficacy through the conduct of appropriate clinical trials. It is necessary to demonstrate that a product will show the established pharmacokinetic profile for the relevant coagulation factors, i.e., *in vivo* recovery and half-life. Proof of efficacy to a clinical endpoint under the various indications sought, e.g., prophylaxis, treatment of episodic bleeds, and surgery, is also needed. Clinical requirements for the assessment of adverse events, such as viral transmission and inhibitor development, demand patient numbers that are considerably larger than is feasible if conventional assurance based on statistical principles is to be generated. Given these limitations, the EMEA has revised its requirements for the clinical efficacy of FVIII and FIX to allow efficacy and adverse event assessment to be reviewed through lower patient numbers than previously required [22]. Similar flexibility has been shown on the need for previously untreated patients (PUPs) and nonbleeding patients, both scarce groups to access. It is still not possible for regulators to fully exempt plasma concentrates from the requirements of clinical studies on the basis of so-called "comparability" with similar products [23]. The level of characterization possible for these products, particularly in relation to potential predictors of efficacy and adverse events, is necessarily limited, and surrogates for clinical studies are not yet available.

While the pressure is on regulators to avoid "over-regulation," there are still some areas of plasma product regulation where provisions could be strengthened. The European system mandates centralized—and rigorous—oversight for these products if they are manufactured using certain biotechnological techniques, e.g., monoclonal antibody affinity chromatography. Products produced with earlier technology are regulated through the "mutual recognition" procedure [24] emanating from review by one authority in a single member state. Considering that these products were developed and placed on the market prior to the modern era of stringent regulation, the maintenance, in individual European states, of hemophilia products that are not reflective of current best practice in the field demands review if patient care and safety are to be ensured.

Postmarket surveillance

Once products are approved and on the market, it is essential that their quality, safety, and efficacy are maintained throughout their market lifetime. This is achieved through postmarket activities, which include:

- maintenance of GMP through regular inspections after the introduction of the products;
- testing of batches prior to release in order to ensure conformance to specifications; and
- appropriate recording and reporting mechanisms for adverse events.

The importance of these measures needs to reflect the fact that novel plasma-derived hemophilia concentrates are rare nowadays, and their presence on the market can be prolonged. The products introduced in the mid-1980s were developed prior to the many additional measures that were subsequently incorporated into the regulatory requirements as more problems were identified. It is therefore reasonable to maintain vigilance with these more recently identified issues in mind, particularly in relation to pathogen safety issues. Appropriate adverse event detection and reporting assists in this. Of more questionable relevance is the role of prerelease testing of all batches introduced into the market by the regulatory authority. It is pertinent that such testing cannot be applied to the primary pressure point of viral safety. Some government agencies in less developed countries attempt to assure viral safety through demanding end-product testing using serologic or molecular techniques to detect viral markers used for blood screening. This approach is scientifically worthless. However, end-product testing for potency still has a role, particularly for products newly developed and released. The vagaries of coagulation factor assay and standards in relation to hemophilia concentrates are well recognized [25] and batch release testing assists in the detection of problems, as exemplified by B-domain-deleted recombinant FVIII (BDD-FVIII) (see below).

Issues related to hemophilia concentrates from recombinant technology

The developments in viral safety over the past 20 years have achieved plasma product safety to a large extent. The robustness of viral inactivation techniques was tested successfully when the West Nile virus (WNV) entered the North American blood supply during 2002 and infected the recipients of fresh blood components but not plasma derivatives [26]. However, the prospect of previously unknown and resistant pathogens became an acute concern in the late 1990s with the emergence of the prionic diseases as possible threats to blood safety [27]. This issue emphasized the importance of continuing to move to alternatives of blood-derived therapeutics when possible.

Essentially, all the principles specified for plasma concentrates are equally applicable and mandatory for recombinant alternatives. The importance of these principles is best demonstrated by reviewing a series of incidents involving recombinant products that demanded regulatory engagement:

- In 2001, a serious breach of GMP forced the FDA to severely restrict the production capacity of a recombinant manufacturer, affecting product supply worldwide [28]. This

breach of GMP had the potential to affect product and patient safety. The importance of maintaining a GMP audit program was emphasized by this incident.

- In 2001, the EMEA restricted the use of recombinant FIX in pediatric patients when postmarket studies required as a condition of European licensure indicated a worrisome variability in the pharmacokinetics of the product in these patients [29].
- In 2003, studies performed in European medicine control laboratories indicated potency problems in a B-domain-deleted (BDD) recombinant FVIII (Refacto®), as a result of a potency misalignment in the initial calibration of the standard of the product [30].

Of particular relevance is the role of prerelease potency testing in detecting the BDD-FVIII problem. This problem would have remained undetected in the absence of such testing, which has been excised from the regulatory framework of the FDA under the consideration that recombinant coagulation products represent "well-specified proteins" whose regulation does not demand the same level of depth and rigor required of plasma products.

The current generation of recombinant products is available in formulations lacking any blood or tissue excipient, and products that have this feature through their manufacture are expected imminently. The only FIX recombinant product—Benefix®—is of this type, and may be described as state of the art in terms of pathogen safety. This product, however, has raised other issues pertinent to the oversight of the recombinant coagulation factors. The differences in higher order structure in this FIX compared with the wild-type molecule have been implicated in the lower *in vivo* recovery of this product [31]. The problem with this product highlights the desirability of regulators and industry reviewing the whole standardization framework for hemophilia products, traditionally derived from the concept of a "biological unit" initially defined as the amount in 1 mL of normal plasma. The lineage of the various standards used in this environment has been reviewed [32]. This author believes that, with the development of pure and recombinant variants of the plasma-derived protein, the adherence to this concept requires review. The issue is well exemplified by the Benefix problem, where the pharmaceutical development based on the biologic unit was unable to predict the lower recovery of the recombinant FIX. Thus, the product development, including clinical development and dosage, for these products, should be based on the concept, now possible, of labeling and dosing using product mass rather than units. This would obviate problems resulting from an inappropriate alignment to concepts and standards based upon the wild-type molecule. The analogy in the area of fibrinolysis, where a truncated tissue plasminogen activator is labeled relative to its own particular standard, suggests the feasibility of developing particular standards for novel molecules. It may be argued that this concept should only be applied for a radical molecular change like deletion of the B domain in FVIII. For that product, the dangers of initially aligning the potency with the wild-type molecule standard were demonstrated by the BDD-FVIII problem referred to above [33]. The use of mass rather than unitage has been successfully applied to the use of recombinant FVIIa in the treatment of inhibitors in hemophilia [34].

Debate continues on the issue of inhibitor risk associated with recombinant products. Some compelling though limited studies indicate that plasma-derived products containing factor VIII associated with von Willebrand factor have a lower inhibitor risk than recombinant products [35]. The European Medicines Agency has been reluctant to endorse this view [36] but has enhanced the warning statement on recombinant products with regard to inhibitor risk [37], while emphasizing the importance of continued postmarket surveillance.

Conclusion

Currently, the regulation of products for hemophilia in less developed economies relies on reference to decisions in the First World authorities. This may not always result in optimal outcomes as most of the hemophilia care in the developing world is through local plasma and cryoprecipitate, which are not subject to the oversight of mainstream regulators. Furthermore, the emergence of companies based outside the developed world and seeking to supply the emerging economies of the developing world with hemophilia concentrates has necessitated new strategies for regulation that are independent of the established frameworks. A minimalist approach developed by the World Federation of Hemophilia is intended to address the needs of less developed countries seeking to ensure optimal outcomes under their own, more limited, circumstances [38]. Overall, the principles used by mainstream agencies and described in this chapter may be applied in all environments seeking to assure the quality of hemophilia care. Applied properly, they can contribute to maintaining the delivery of a form of therapy that is nowadays among the safest in therapeutic practice.

References

1 Hoots WK. Comprehensive care for hemophilia and related inherited bleeding disorders: Why it matters. *Curr Hematol Rep* 2003; **2**: 395–401.
2 European Council Directive 75/319/EEC. Available at http://www.europa.eu.int (accessed on 15 November 2009).
3 European Council Directive 89/381/EEC. Available at http://www.europa.eu.int (accessed on 15 November 2009).
4 Farrugia A. The regulatory pendulum in transfusion medicine. *Trans Med Rev* 2002; **16**: 273–82.
5 Plasma Protein Therapeutics Association. Standards for members under the QSEAL Program. Available at: http://www.plasmatherapeutics.org/en/qualitysafetyqseal.cfm (accessed on 15 November 2009).
6 Farrugia A. Evolving perspectives in product safety for haemophilia. *Haemophilia* 2002; **8**: 236–43.

7 Lloyd S. The preparation of single donor cryoprecipitate. Available at: http://www.wfh.org/Content_Documents/FF_Monographs/FF2_Cryo_V6.doc (accessed on 15 November 2009).

8 Srivastava A. Factor replacement for haemophilia—should cryoprecipitate be used? *Haemophilia* 1999; **5**: 301–5.

9 Evatt, Austin H, Leon G, *et al.* Haemophilia therapy: Assessing the cumulative risk of HIV exposure by cryoprecipitate. *Haemophilia* 1999; **5**: 295–300.

10 Keeling DM, Luddington R, Allain JP, *et al.* Cryoprecipitate prepared from plasma virally inactivated by the solvent detergent method. *Br J Haematol* 1997; **96**: 194–7.

11 McLeod BC, Sassetti RJ, Cole ER, Scott JP. A high-potency, single-donor cryoprecipitate of known factor VIII content dispensed in vials. *Ann Intern Med* 1987; **106**: 35–40.

12 Burnouf T, Caron C, Radosevich M, Goubran HA, Goudemand J, El-Ekiaby M. Properties of a concentrated minipool solvent-detergent treated cryoprecipitate processed in single-use bag systems. *Haemophilia* 2008; **14**: 956–62.

13 Code of Federal Regulations of the United States. Available at: www.fda.gov (accessed on 15 November 2009).

14 European Commission. Directive 2002/98/EC of the European parliament and of the council of 27 January 2003 setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending directive 2001/83/EC. Available at: http://europa.eu.int/eur-lex/en/dat/2003/l_033/l_03320030208en00300040.pdf (accessed on 15 November 2009).

15 Council of Europe. Guide to the preparation, use and quality assurance of blood components—8th edition (2002). Available at: http://book.coe.int/GB/CAT/LIV/HTM/l1838.htm (accessed on 15 November 2009).

16 Pharmaceutical Inspection Convention (2003) Guide to Good Manufacturing Practice for medicinal products. Annex 14 Manufacture of products derived from human blood or human plasma. Available at: http://www.picscheme.org/docs/pdf/PE%20009-1%20GMP%20Guide%20September%202003.pdf#PE%20009-1 (accessed on 15 November 2009).

17 Williams PE, Yap PL, Gillon J, *et al.* Transmission of non-A, non-B hepatitis by pH4-treated intravenous immunoglobulin. *Vox Sang* 1989; **57**: 15–18.

18 Therapeutic Goods Administration. Blood regulator takes action on South Australian Blood Service—Friday 12th September 2003. Available at: http://www.health.gov.au/tga/docs/html/mediarel/mrsablood.htm (accessed on 15 November 2009).

19 Plasma Protein Therapeutics Association. Voluntary Standard for Intermediates. Available at: http://www.pptaglobal.org/librarydocs/PPTAIntermediatesStandard.pdf (accessed on 15 November 2009).

20 Committee for Proprietary Medicinal Products. Note for Guidance on Virus Validation Studies: The design, contribution and interpretation of studies validating the inactivation and removal of viruses. Available at: http://www.emea.eu.int/pdfs/human/bwp/026895en.pdf (accessed on 15 November 2009).

21 International Conference on Harmonisation. General Information on the Common Technical Document. Available at: http://www.ich.org/WORD/CTD%20General%20Info.ppt (accessed on 15 November 2009).

22 Committee for Proprietary Medicinal Products. Note for Guidance on the clinical investigation of human plasma derived Factor VIII and IX products. Available at: http://www.emea.eu.int/pdfs/human/bpwg/019895en.pdf (accessed on 15 November 2009).

23 Center for Biologics Evaluation and Research. Comparability Studies for Human Plasma-Derived Therapeutics—Workshop on Friday, May 31, 2002. Available at: http://www.fda.gov/cber/minutes/plasma 053102.htm (accessed on 15 November 2009).

24 European Commission 2001. Proposal for a regulation of the European Parliament and the Council laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Agency for the Evaluation of Medicinal products. Available at: http://europa.eu.int/eur-lex/en/com/pdf/2001/en_501PC0404_01.pdf (accessed on 15 November 2009).

25 Barrowcliffe TW, Mertens K, Preston FE, Ingerslev J. Laboratory aspects of haemophilia therapy. *Haemophilia* 2002; **8**: 244–9.

26 Pealer LN, Marfin AA, Petersen LR, *et al.* Transmission of West Nile virus through blood transfusion in the United States in 2002. *N Engl J Med* 2003; **349**: 1236–45.

27 Brown P. Creutzfeldt–Jakob disease: Blood infectivity and screening tests. *Semin Hematol* 2001; **38**: 2–6.

28 Center for Biologics Evaluation and Research. Warning letter to Carol M. Moore, Vice President, Quality Assurance/Regulatory Affairs, Bayer Corporation, Biologics Product Division. Available at: http://www.fda.gov/foi/warningletters/g1575d.pdf (accessed on 15 November 2009).

29 European Medicines Evaluation Agency. Public Statement on Benefix (nanacog alfa). Intensive post-marketing surveillance for all new patients—new clinical trials. 04 October 2001. Available at: http://www.eudra.org/humandocs/Humans/EPAR/Benefix/Benefix.htm (accessed on 15 November 2009).

30 Hubbard AR, Sands D, Sandberg E, Seitz R, Barrowcliffe TW. A multi-centre collaborative study on the potency estimation of Refacto. *Thromb Haemost* 2003; **90**: 1088–93.

31 White GC 2nd, Beebe A, Nielsen B. Recombinant factor IX. *Thromb Haemost* 1997; **78**: 261–5.

32 Barrowcliffe TW. Clotting factor concentrates in clinical practice. Standardization and assay. *Semin Thromb Haemost* 1993; **19**: 73–9.

33 Farrugia A. Potency assessment of the new generation of coagulation factor concentrates—time for a new paradigm? *Thromb Haemost* 2003; **90**: 968–70.

34 Negrier C, Lienhart A. Overall experience with NovoSeven. *Blood Coagul Fibrinolysis* 2000; **11** (Suppl. 1): S19–24.

35 Goudemand J, Rothschild C, Demiguel V, Vinciguerrat C, Lambert T, Chambost H, Borel-Derlon A, Claeyssens S, Laurian Y, Calvez T; FVIII-LFB and Recombinant FVIII study groups. Influence of the type of factor VIII concentrate on the incidence of factor VIII inhibitors in previously untreated patients with severe hemophilia A. *Blood* 2006; **107**: 46–51.

36 European Medicines Evaluation Agency. Review of recombinant Factor VIII products and inhibitor development. Available at: http://www.emea.europa.eu/pdfs/human/press/pus/33131605en.pdf (accessed on 15 November 2009).

37 European Medicines Evaluation Agency. EMEA completes the review of recombinant Factor VIII products and inhibitor development. Available at: http://www.emea.europa.eu/pdfs/human/press/pus/31022507en.pdf (accessed on 15 November 2009).

38 Farrugia A. Guide for the assessment of clotting factor concentrates for the treatment of haemophilia 2003. Available at: http://www.wfh.org/Content_Documents/Other_Publications/Reg_Guide_Eng.pdf (accessed on 15 November 2009).

27 Joint replacement

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Introduction

Hemophilia care has steadily improved over the years and especially so during the last decade. The routine use of prophylactic treatment has undoubtedly resulted in a significant improvement in the lifestyle, quality of life, and life expectancy of these patients, and bodes well for the future. However, despite our best efforts there is still a group of young adults who have a severe degree of knee joint destruction as a result of repeated articular bleeding episodes during their early years.

Total joint replacement has transformed the quality of life for countless thousands of patients worldwide but there has been a reluctance to offer this operation to younger patients. However, the results of joint replacement in patients under 55 are generally equivalent to those in the older population and our previous conservative approach was perhaps flawed.

The combined experiences of joint replacement over the past 20 years have been encouraging, with significant improvements in measurable outcome parameters, reduction in spontaneous hemarthroses, and improvements in quality of life.

We believe that total joint replacement is a safe and effective procedure in the management of hemophilic joint arthropathy. The latest techniques using continuous infusion and recombinant factor replacement have gone a long way to reducing the complications rate and to achieving results that match those of the general population.

Joint replacement surgery is never a simple undertaking in someone with hemophilia; it requires aggressive factor replacement pre- and post surgery, a long hospital stay, and considerable rehabilitation. Fortunately, the majority of our patients are healthy, motivated, and well prepared for the operation that they may be facing. They are young men in the prime of their life professionally and physically whose quality of life is affected by a single destroyed joint.

The major objective of total joint replacement is to reduce the level of pain in the affected joint. This will have beneficial effects on the adjacent joints. In addition, there is a significant reduction in the frequency and number of joint bleeds, which all adds up to an increase in both function and mobility.

The operation should only take place in a recognized center with appropriate hematologic back-up. This is not surgery for the occasional operator.

At the Royal Free Hospital in London there are now approximately 1500 patients with inherited bleeding disorders registered at the unit, of whom some 200 are affected with severe hemophilia. We have adopted a team approach in the management of our patients. We hold a dedicated monthly combined clinic run by the hematologist and the orthopedic surgeon, with other key members involved, including the physiotherapist, nurse, and counselor. The clinic is held in a relatively open format and thus information and experiences are traded and shared between patients, who are often valuable sources of advice, inspiration, and reassurance.

Total joint replacement now forms the bulk of our surgery. Our experience now extends to 95 total joint replacements in 55 patients. 65 total knee replacements (TKRs) have been performed in 44 patients (nine simultaneous bilateral, and five revisions), 24 total hip replacements (THRs) in 17 patients (two bilateral and two revisions), two shoulder replacements, and five elbows.

In the past, there were valid concerns in performing joint replacement in human immunodeficiency virus (HIV)-positive patients. However, modern treatment for HIV has reduced the risk of postoperative infection, and in a recent review Habermann *et al.* [1] concluded that total joint replacement in hemophilic patients with or without HIV was safe, and that the functional outcome does not differ from an HIV-negative population both in the TKR and THR groups. In our experience, however, there are possible complicating factors related to the increased potential for bleeding in those patients on combination therapy, particularly the protease inhibitors.

Total knee replacement

The knee is the most common joint affected in hemophilia (approximately 50%) and despite our best efforts there is still a group of young adults who have a severe degree of knee joint destruction as a result of repeated articular bleeding episodes during their early years. This in turn leads to the onset of pain and significant functional disability at a time when the patient requires the best possible quality of life.

The indications for operation are primarily disabling pain that is unresponsive to medical treatment. Deformity and poor

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functional range of motion, particularly a severe flexion contracture of the knee, are relative indications and may in themselves justify joint replacement. However, joint contractures and flexion deformity pose various surgical challenges for the surgeon.

The main question remains as to when one should offer such surgery given the widely held belief that relative youth is a contraindication. This may, in fact, be true of the "normal population" with single joint involvement whose demands and expectations may be unrealistic, but this is generally not the case in the present population of hemophilic patients who frequently have other joint involvement. With this in mind and armed with the knowledge of encouraging reports of excellent long-term results of total knee replacement in the <55 age group, knee replacement represents an appropriate procedure.

The early literature was full of pessimism, reporting poor results and in particular a high incidence of both significant bleeding episodes and deep joint infection, up to 17% in some series [2–4].

The biggest problem arose between 1979 and 1985 when some 80–90% of the hemophilic population in the developed world became infected with the HIV virus. These patients were shown to be more susceptible to infection and in 1993 it was shown that there was a high risk of secondary infection in HIV-positive patients and that surgery should be reserved for a carefully selected group of patients [5]. Their experience was confirmed by Weidel *et al.* [6] and later by Ragni *et al.* [7] who reported a 30% incidence of infection in a series of 27 total knee arthroplasties in HIV-positive patients in stark contrast to an infection rate of 1–2% in the nonhemophilic population and concluded that when offering such surgery to HIV-infected patients there must be a very careful analysis of the risk–benefit ratio.

Data from the Royal Free group was, however, more encouraging with Birch [8], and later, Philips *et al.* [9], reporting that there was no evidence to suggest that major surgery had any adverse effects on the rate of decline in the CD4 count, the onset of AIDS nor the mortality rate, and that the outcome of all the TKRs were excellent or good with no acute infections.

Total joint replacement in HIV-positive patients with hemophilia still carries a potential risk. The pooled data therefore suggest that total knee arthroplasty in hemophilic patients carries with it an increased risk of postoperative infection in comparison with nonhemophilic patients. Those patients at particular risk are the HIV-positive hemophilic patients whose CD4 count is less than 200 cells/mm [10,11]. We believe that, nonetheless, total knee arthroplasty is a surgical procedure that should be considered for selected cases.

The operation consists of a standard resurfacing TKR combined with an adequate synovectomy. Details of the procedure are covered in other texts; however, one must appreciate that the surgery itself is not always straightforward given the high incidence of joint deformity and soft tissue contractures and that accordingly some experience of revision knee replacement is a distinct advantage.

It should be borne in mind that, postoperatively, these patients often require higher doses of opiates for longer periods than patients undergoing TKR for straightforward osteoarthritis.

There is little doubt nowadays that total knee arthroplasty represents a safe and effective procedure in the management of hemophilic joint arthropathy. The latest techniques using continuous infusion and recombinant factor replacement have gone a long way to reducing the complications rate and to achieving results that match those of a similar nonhemophilic population especially with regard to pain relief, restoration of function, and, importantly, an improvement in the quality of life.

Rodriguez-Merchan and Wiedel [12] reported their combined experience of 37 total knee arthroplasties performed on 26 men between March 1975 and November 1995. Of this group, 17 (46%) were HIV positive, of whom nine died from complications of AIDS. Overall, 84% of this group was classed as good to excellent, 8% fair, and 8% poor according to their HSS (Hospital for Special Surgery) rating. Importantly, the greatest improvements were with regard to pain and function, with a less marked improvement with respect to range of motion. The incidence of hemarthroses has dropped from a mean of 5.5 per annum to 1.2.

The experience at the Royal Free Hospital is very similar [13]. Over the period 1983–2008, 65 primary TKRs were performed in 44 patients. The mean HSS scores have improved from 30 preoperatively to 79.5 post operatively, with 86% of patients being rated excellent or good, 5% fair, and 9% poor. There have been no major complications and only one case of late infection and one unusual case of prolonged postoperative bleeding, which was subsequently shown to be a result of an aneurysm of the lateral inferior geniculate artery [14].

We have also undertaken seven simultaneous bilateral TKRs during the past 7 years. Our results for this procedure are identical to unilateral TKR or staged bilateral TKR, with no increase in the rate of complications, or prolongation of the hospital stay. There are obvious financial advantages, since only one hospitalization is required and significantly the factor replacement requirements are the same as for a single procedure.

Elbow replacement

The elbow is the second most common site for arthropathy in the hemophilic patient. Destructive changes occur insidiously as it is not a classical weight-bearing joint and early limitations of flexion and extension seldom interfere with overall function [15].

Various operations have been described in the use of elbow arthropathy associated with hemophilia. These operations include synovectomy, simple excision of the radial head combined with joint debridement, excision arthroplasty, arthrodesis, and silastic interposition arthroplasty [15–19]. Excision of the radial head combined with synovectomy has resulted in

consistently good results with reduction in pain, an increased range of motion, and a reduction in the frequency of joint bleeds.

In 1990 the group from St Thomas' Hospital in London reported their experience of 13 elbows affected by severe hemophilic arthropathy and treated by silastic interposition arthroplasty [20]. The severity of pain, the frequency and severity of spontaneous hemorrhage and the range of movement were much improved and needed less factor replacement. Three elbows were revised; one for infection and two because of fragmentation of the silastic sheet with good restoration of function following revision.

The actual incidence of joint replacement, however, is likely to be low given that Bajekal reported that 81% of hemophilic patients suffered recurrent elbow bleeds but reported a low incidence of total joint replacement in the same group [21].

Whilst total joint replacement has been well described for the hip, knee, and shoulder in hemophilia there have been few reports concerning total elbow replacement. Most reports have been restricted to isolated case reports [22,23]. The first report by Luck and Kasper [24] reviewed the 20-year results of a total of 168 surgical procedures carried out for hemophilic arthropathy but included only two total elbow replacements, one of which became infected. Kasten and Skinner [25], in their large series of total elbow replacements, described only two cases of hemophilia—one primary and a further revision—likewise described by Chatelot *et al.* [26].

The largest series to date was recently reported from the Oxford group and concerned seven elbow replacements in five patients with severe hemophilia A [27]. All patients demonstrated excellent relief of pain and improvement of function. There was one failure because of infection in an immunocompromised patient coinfected with HIV and hepatitis C. The patients were followed for a minimum of 25 months and implants varied from unconstrained (Kudo or Souter–Strathclyde) to the more constrained Coonrad–Morrey joint replacements. There were three major postoperative complications, one ulnar nerve palsy, one axillary vein thrombosis and one patient who developed late infection requiring excision arthroplasty.

Overall, the results of the patients in this group were excellent in the short to medium term, and they concluded that total elbow replacement is both feasible and useful in patients with severe hemophilic arthropathy.

Ankle replacement

The ankle joint is third most commonly affected joint accounting for 14.5% of recorded bleeds. The pattern of bleeding varies according to age, with the ankle most commonly affected during the second and third decades of life. Once the joint has been subjected to repeated bleeds it becomes more vulnerable and ultimately becomes a target joint with relentless deterioration of function and comfort in the hind foot [28].

Surgical intervention is only considered when conservative measures have failed. In patients with synovitis, recurrent hemarthroses and with congruent joint surfaces synovectomy, either open, arthroscopic, or chemical (synoviorthesis) may decrease pain and prevent recurrent bleeding. In more advanced cases, joint debridement and excision of osteophytes (O'Donoghue procedure) can restore some joint motion [29], especially dorsi-flexion. In our experience, though, any benefit is relatively shortlived and further surgery has become necessary within 5 years [30,31].

Pearce *et al.* [32] reported their experience of supramalleolar varus osteotomy on seven ankles (in six patients) for hemophilic arthropathy and secondary valgus deformity. The operation reduced pain and the frequency of intra-articular bleeding while preserving joint function for a mean of 9 years. It was considered that this procedure offered a good alternative to the more commonly used surgical option of arthrodesis.

In patients with severe pain and end-stage arthropathy, who have failed conservative treatment, an ankle arthrodesis may be indicated. The surgery is intended to produce a more comfortable hind-foot unit with better ambulation and a reduced bleeding tendency. There are relatively few reports of ankle arthrodesis in the literature but our experience is that the procedure is well tolerated and success can generally be assured provided care is taken over the final position of the talus and hind foot [18,30,31,33].

Total ankle replacement has been shown to have good medium-term results in low-demand patients with rheumatoid arthritis, but poorer results in patients with osteoarthritic degenerative ankle disease. Luck [18] reported a case of ankle replacement in hemophilia and although the patient did well, the author concluded that "ankle and sub-talar joint are best treated by arthrodesis rather than by arthroplasty."

Prosthetic design, however, has improved and the results are now more encouraging. The literature, however, is still limited to case reports and small series [34,35]. Van der Heide [35] reported a series of five total ankle replacements in three patients with a median follow up of 4.3 years (range 1–8.7). All prostheses were still in place and did not show any signs of loosening. Clinical scores showed a good to excellent result. He concluded that, in his experience, total ankle replacement in patients with bleeding disorders showed promising results.

However, the place for ankle replacements remains uncertain in the present climate, given the poor long-term results in other series, particularly when contrasted with the good results of ankle (talocrural) arthrodesis.

Total hip replacement

The true incidence of hemophilic arthropathy of the hip is relatively rare, accounting for only approximately 4% of patients [36]. Nonetheless, degenerative disease of the hip represents a significant volume of the orthopedic surgeon's workload and it is therefore perhaps more pertinent to con-

sider patients with hemophilia as having osteoarthritis of the hip rather than true hemophilic arthropathy.

Unlike knee replacement, the results of THR in hemophilia remain relatively poor in the medium to long term. The common complications of THR are those common to the uncomplicated procedure. Deep infection remains the most significant and indeed serious complication, and it has been estimated that the incidence of infection in the hemophiliac is approximately three times the anticipated incidence with a high infection rate and a 33% incidence of aseptic loosening. It has been proposed that this could be related to coexisting knee and ankle involvement resulting in a tendency for the patient to walk with a stiff-legged gait so subjecting the hip to additional stress, which, with the passage of time, can cause the prosthetic hip to loosen.

Luck and Kasper [24] published the first significant series of THRs in hemophilic patients in 1989. This was a series of 13 patients over a 20-year period with a 60% failure rate largely owing to infection. The Mayo Clinic reported a series of 12 hip replacements with only six rated excellent or good at 5 years' follow-up [37]. Six hips were rated poor or failures, with four being painful with evidence of gross loosening and infection affecting the other two.

The largest published series to date is that of Nelson *et al.* [36], who reported a series of 39 THRs with a mean age of 48 years and mean follow-up of 7.6 years. Of the 22 available for review, five had already been revised and a further three were awaiting revision representing a failure rate of at least 30%. Similar results were reported by Lofqvist [38], with five out of 13 hips having failed by 6 years and Heeg [39], with a 33% failure rate at 9 years.

Kelly *et al.* [40] set out to determine the true incidence of hemophilic arthropathy in hip patients and performed a multicenter analysis. They looked at 34 THRs in four major hemophilia centers in the USA. The mean duration of follow-up was 8 years, with a minimum of 2 years. Four patients were HIV positive. There were no early infections after 34 replacements, but there were three late infections possibly related to HIV. As with earlier series, though, the aseptic loosening rate was 21% of the 28 cemented femoral components and 23% of the 26 cemented acetabular components.

Recent data from our own institution are more encouraging in a report of 34 hip replacements for painful end-stage hemophilic arthropathy in 24 patients over a 23-year period [41]. Of the 21 patients available for review there was one case of deep infection presenting 3 years after initial surgery, and three cases of aseptic loosening at a mean time to failure of 7.3 years. Interestingly, all four cases had hepatitis C virus infection, but were HIV negative. All cases were successfully revised with no further complications. One patient developed heterotopic bone formation 3 years postoperatively, which required excision [42]. There were three cases of superficial wound infections which responded to antibiotic treatment. There were no other complications.

As with TKR, there are undoubtedly benefits to the patient following THR, but the objective outcome measures and the incidence of complications is higher than in the normal population, possibly owing to the relatively young population (mean age 51). Despite the increased risks there seems to be general agreement that THR is an appropriate operation for disabling arthropathy in hemophilic patients.

Total shoulder replacement

The shoulder has not been considered to be a major problem in hemophilia [43]. Indeed, in a recent review article Gilbert referred to the shoulder as the "neglected joint" [44]. This could be because of the unique anatomic arrangement of the shoulder girdle with movement taking place at both glenohumeral and scapulothoracic levels. This double articulation will thus still permit a functional range of "shoulder" movement even in the absence of any glenohumeral motion. Nonetheless, hemophilic arthropathy is less common in the shoulder than either the elbow or the knee but can lead to significant pain and loss of function.

The reported incidence of symptomatic hemophilic arthropathy of the shoulder is relatively rare. Pettersson reported the results of two studies undertaken at the Malmö Haemophilia Centre in the early 1960s and again in the early 1990s [43]. In the early study, incidence of symptomatic shoulder arthropathy was 13% of patients, one-third of whom were below the age of 30. In the later study, the incidence was approximately the same (16%, but no patients were below the age of 35), which led to the conclusion that isolated shoulder hemorrhages and shoulder arthropathy in hemophiliacs with prophylactic treatment was uncommon. It is likely, of course, that this pattern will be reflected in other joints now that prophylactic treatment is commonly practiced.

Shoulder problems have traditionally been dealt with using nonoperative measures, trying to minimize the prevalence for bleeding. Radioactive or chemical synoviorthesis have been performed, and synovectomy, either open or arthroscopic, is now becoming more common. Fusion of the shoulder can be effective in converting a painful, stiff joint into a painless stiff joint [24].

However, if conservative management fails to relieve severe, unremitting shoulder pain in the presence of underlying arthropathy, it may be reasonable to consider shoulder arthroplasty. Total shoulder replacement remains a relatively uncommon procedure undertaken in hemophilia with sporadic case reports only. Luck and Kasper [24] reported three patients with satisfactory results with regard to both pain relief and range of motion. Greene [45] recorded a satisfactory outcome in a single patient who underwent a total shoulder replacement who later died of the effects of HIV. Phillips *et al.* [23] reported the successful outcome of a case of ipsilateral shoulder and elbow replacement in a patient with hemophilia B. Dalzell [46] reported three hemiarthroplasties on two men

with hemophilia. In each case there was a decrease in pain, and an increase in range of motion and function.

Now that prophylactic factor treatment programs are becoming the norm, the incidence of bleeding into the shoulder and subsequent arthropathy is becoming less frequent. It is therefore probable that fewer patients will present with significant shoulder symptoms and that the demand for shoulder intervention, and shoulder replacement in particular, will remain minimal for the foreseeable future.

Conclusion

It would therefore seem that, in the appropriate setting, joint replacement surgery should be the treatment of choice in hemophilic patients suffering from severe arthropathy and disability. There can be little doubt that a successful implant provides considerable benefits in the majority of hemophilic patients, with marked pain relief and improvement in function. However, the increased risk of infection and noninfective complications remains a cause for concern.

Thus, before embarking upon a potentially dangerous procedure the orthopedic surgeon should consider the risks and benefits, very carefully taking into account the patient's age, ambitions, life expectancy, and immunologic status. The patient should be managed in a dedicated hemophilia center where a comprehensive team approach can be provided.

The risk of HIV infection through contaminated blood products has significantly diminished with time since coagulation factor concentrates have been subject to virucidal agents, and in the UK there have been no new HIV infections in hemophiliacs since 1986. In addition, the introduction of recombinant factor replacement in 1998 has been a major development, particularly in removing the unacceptably high risk of viral transmission (HIV, hepatitis B and C, variant Creutzfeldt–Jakob disease).

Patients with HIV infection now are living longer as a result of the improvements in medication and combination therapy, and results of joint replacement in HIV coinfected patients do not seem to differ from noninfected cohorts. There still remains a question as to the effect of combination therapy, particularly protease inhibitors, on hemostasis and there is a degree of uncertainty around the timing of withdrawing treatment prior to surgery.

For hemophilic patients in the developing world there is accumulating evidence that better prophylaxis in childhood and adolescence is leading to a marked reduction in hemophilic arthropathy. Hopefully, therefore, the need for operative intervention will also diminish. Ultimately, of course, gene therapy may cure the disease for ever.

References

1 Habermann B, Eberhardt C, Kurth AA. Total joint replacement in HIV positive patients. *J Infect* 2008; **57**: 41–6.

2 Lachiewicz PF, Inglis AE, Insall JN, Sculco TP, Hilgartner MW, Bussel JB. Total knee arthroplasty in hemophilia. *J Bone Joint Surg Am* 1985; **67**: 1361–6.

3 Karthaus RP, Novakova IR. Total knee replacement in haemophilic arthropathy. *J Bone Joint Surg Br* 1988; **70**: 382–3.

4 Thomason HC, III, Wilson FC, Lachiewicz PF, Kelley SS. Knee arthroplasty in hemophilic arthropathy. *Clin Orthop* 1999; **360**: 169–73.

5 Gregg-Smith SJ, Pattison RM, Dodd CAF, Duthie RB. Septic arthritis in haemophilia. *J Bone Joint Surg Br* 1993; **75**: 368–70.

6 Wiedel JD, Luck JV, Gilbert MS. Total knee arthroplasty in the patient with haemophilia; evaluation and long term results. In: Gilbert MS, Greene WB (eds.) *Muscelo-sketelal problems in Hemophilia*. New York: National Hemophilia Foundation, 1989: 152–7.

7 Ragni MV, Crossett LS, Herndon JH. Postoperative infection following orthopaedic surgery in human immunodeficiency virus-infected hemophiliacs with CD4 counts < or =200/mm^3. *J Arthroplasty* 1995; **10**: 716–21.

8 Birch NC, Ribbans WJ, Goldman E, Lee CA. Knee replacement in haemophilia. *J Bone Joint Surg Br* 1994; **76**: 165–6.

9 Phillips AM, Sabin CA, Ribbans WJ, Lee CA. Orthopaedic surgery in hemophilic patients with human immunodeficiency virus. *Clin Orthop* 1997; **343**: 81–7.

10 Hicks JL, Ribbans WJ, Buzzard B, *et al.* Infected joint replacements in HIV-positive patients with haemophilia. *J Bone Joint Surg Br* 2001; **83**: 1050–4.

11 Rodriguez-Merchan EC, Wiedel JD. Total knee arthroplasty in HIV-positive haemophilic patients. *Haemophilia* 2002; **8**: 387–92.

12 Rodriguez-Merchan EC, Wiedel JD. Total knee arthroplasty in haemophilia. In: Rodriguez-Merchan EC, Goddard NJ, Lee CA (eds.) Musculoskeletal Aspects of Haemophilia. Oxford: Blackwell, 2000: 78–84.

13 Goddard NJ, Rodriguez-Merchan EC, Wiedel JD. Total knee replacement in haemophilia. *Haemophilia* 2002; **8**: 382–6.

14 Mann HA, Goddard NJ, Lee CA, Brown SA. Periarticular aneurysm following total knee replacement in hemophilic arthropathy. *J Bone Joint Surg Am* 2003; **85**: 2437–40.

15 Gilbert MS, Glass KS. Hemophilic arthropathy of the elbow. *Mt Sinai J Med* 1977; **44**: 389–96.

16 Kay L, Stainsby D, Buzzard B. The role of synovectomy in the management of recurrent haemarthroses in haemophilia. *Br J Haematol* 1981; **49**: 53–60.

17 Le Balc'h T, Ebelin T, Laurian Y. Synovectomy of the elbow in young hemophilic patients. *J Bone Joint Surg Am* 1987; **69**: 264–9.

18 Luck JV, Jr. Surgical management of advanced hemophilic arthropathy. *Prog Clin Biol Res* 1990; **324**: 241–56.

19 Rodriguez-Merchan EC, Galindo E, Magallon M, Cage J, Villar A. Resection of the radial head and partial open synovectomy of the elbow in the young adult with haemophilia. *Haemophilia* 1995; **1**: 262–6.

20 Butler-Manuel PA, Smith MA, Savidge GF. Silastic interposition for haemophilic arthropathy of the elbow. *J Bone Joint Surg Br* 1990; **72**: 472–4.

21 Bajekal RA, Phillips AM, Ribbans WJ. Elbow arthropathy in haemophilia. *J Bone Joint Surg Br* 1996; **1**(Suppl.): 15.

22 Beeton K, Rodriguez-Merchan EC, Alltree J. Total joint arthroplasty in haemophilia. *Haemophilia* 2000; **6**: 474–81.

23 Phillips AM, Ribbans WJ, Goddard NJ. Ipsilateral total shoulder and elbow prosthetic replacement in a patient with severe haemophilia B. *Haemophilia* 1995; **1**: 270–3.

24 Luck JV, Kasper C. Surgical management of advanced haemophilic arthopathy. *Clin Orthop* 1989; **242**: 60–82.
25 Kasten MD, Skinner HB. Total elbow arthroplasty. An 18-year experience. *Clin Orthop* 1993; **290**: 177–88.
26 Chantelot C, Feugas C, Ala ET, Migaud H, Gueguen G, Fontaine C. [Kudo non-constrained elbow prosthesis for inflammatory and hemophilic joint disease: Analysis in 30 cases]. *Rev Chir Orthop Reparatrice Appar Mot* 2002; **88**: 398–405.
27 Chapman-Sheath PJ, Giangrande P, Carr AJ. Arthroplasty of the elbow in haemophilia. *J Bone Joint Surg Br* 2003; **85**: 1138–40.
28 Gamble JG, Bellah J, Rinsky LA, Glader B. Arthropathy of the ankle in hemophilia. *J Bone Joint Surg Am* 1991; **73**: 1008–15.
29 O'Donoghue DH. Impingement exostoses of the tibia and talus. *J Bone Joint Surg Am* 1957; **39**: 835–42.
30 Ribbans WJ, Phillips AM. Hemophilic ankle arthropathy. *Clin Orthop* 1996; **328**: 39–45.
31 Goddard NJ. Haemophilic arthropathy of the ankle. In: Rodriguez-Merchan EC (ed) *The Haemophilic Joints: New Perspectives*. Oxford: Blackwell, 2003: 171–5.
32 Pearce MS, Smith MA, Savidge GF. Supramalleolar tibial osteotomy for haemophilic arthropathy of the ankle. *J Bone Joint Surg Br* 1994; **76**: 947–50.
33 MacNicol MF, Ludlam CA. Does avascular necrosis cause collapse of the dome of the talus in severe haemophilia? *Haemophilia* 1999; **5**: 139–42.
34 Radossi P, Bisson R, Munari F, *et al*. Total ankle replacement for end-stage arthropathy in patients with haemophilia. *Haemophilia* 2008; **14**: 658–60.
35 van der Heide HJ, Novakova I, Waal Malefijt MC. The feasibility of total ankle prosthesis for severe arthropathy in haemophilia and prothrombin deficiency. *Haemophilia* 2006; **12**: 679–82.
36 Nelson IW, Sivamurugan S, Latham PD, Matthews J, Bulstrode CJ. Total hip arthroplasty for hemophilic arthropathy. *Clin Orthop* 1992; **276**: 210–13.
37 Stauffer RN. Hemophilia. In: Morrey B (ed.) *Joint Replacement Arthroplasty*. Mayo Foundation, 1991: 759–64.
38 Lofqvist T, Sanzen L, Petersson C, Nilsson IM. Total hip replacement in patients with haemophilia. *Acta Orthop Scand* 1996; **67**: 321–4.
39 Heeg M, Meyer K, Smid WM, Van Horn JR, Van der MJ. Total knee and hip arthroplasty in haemophilic patients. *Haemophilia* 1998; **4**: 747–51.
40 Kelley SS, Lachiewicz PF, Gilbert MS, Bolander ME, Jankiewicz JJ. Hip arthroplasty in hemophilic arthropathy. *J Bone Joint Surg Am* 1995; **77**: 828–34.
41 Miles J, Rodriguez-Merchan EC, Goddard NJ. The impact of haemophilia on the success of total hip arthroplasty. *Haemophilia* 2008; **14**: 81–4.
42 Mann HA, Choudhury MZ, Lee CA, Goddard NJ. Heterotropic bone formation as a complication of elective joint replacement in haemophilic patients—a case report and literature review. *Haemophilia* 2006; **12**: 672–5.
43 Petersson CJ. The hemophilic shoulder. In: Rodriguez-Merchan EC (ed.) *The Haemophilic Joints; New Perspectives*. Oxford: Blackwell, 2003: 163–6.
44 Gilbert MS, Klepps S, Cleeman E, Cahlon O, Flatow E. The shoulder: A neglected joint. *Int Monitor Haemophilia* 2002; **10**: 3–5.
45 Greene WB, DeGnore L, White G. Orthopaedic procedures and prognosis in hemophilic patients who are sero-positive for human immunodeficiency virus. *J Bone Joint Surg Am* 1990; **72**: 2–11.
46 Dalzell R. Shoulder hemiarthroplasty to manage haemophilic arthropathy: Two case studies. *Haemophilia* 2004; **10**: 649–54.

28 Synoviorthesis in hemophilia

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Introduction

A synoviorthesis consists of the intra-articular injection of a certain material with the aim of "stabilizing" (orthesis) the synovium of a joint (synoviorthesis). There are two basic types of procedures for synovial control: medical synovectomy (or synoviorthesis) and surgical synovectomy (open or arthroscopic). It is commonly accepted today that synoviorthesis is the procedure of choice, and that surgical synovectomy should be performed only if a number of consecutive synoviortheses fail to stop or diminish the frequency of recurrent hemarthrosis [1–7]. Thus, the main indication for a synoviorthesis in a hemophilic joint is chronic hypertrophic synovitis and recurrent bleeding.

It is well known that in hemophilia, one or several joints (elbows, knees, and ankles) tend to bleed (hemarthrosis), beginning during an early age of 2–5 years. The synovium is only able to reabsorb a small amount of intra-articular blood; if the amount of blood is excessive, the synovium will hypertrophy as a compensating mechanism, so that eventually the affected joint will show an increase in size of the synovium: so-called hypertrophic chronic hemophilic synovitis. The hypertrophic synovium is very richly vascularized, so that small injuries will easily make the joint rebleed. The final result will be the classic vicious cycle of hemarthrosis–synovitis–hemarthrosis.

If both phenomena are not controlled, they will eventually result in cartilage and bone damage visible on radiographs (chronic hemophilic arthropathy). All the aforementioned features are caused by the congenital coagulation deficiency of factor VIII (FVIII) (hemophilia A) or factor IX (FIX) (hemophilia B). Continuous prophylaxis from 2 to 18 years will significantly decrease the number of hemarthroses and, therefore, the risk of synovitis will also be diminished. On-demand treatment of hemarthroses usually does prevent the development of chronic synovitis. A very serious complication of substitutive treatment, however, is the development of an inhibitor. In such a circumstance, the frequency and severity of hemarthroses will be more intense, producing a severe degree of synovitis.

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Indication of synoviorthesis

The main indication of synoviorthesis is chronic hypertrophic synovitis associated with recurrent hemarthroses that does not respond to hematologic treatment. Synoviorthesis should be performed under factor coverage to avoid the risk of rebleeding during the procedure. In patients with inhibitors, synoviorthesis can also be performed with minimal risk. In fact, the procedure is especially indicated in patients with inhibitors owing to its ease of performance and low rate of complications.

When a child starts to suffer from recurrent hemarthroses that cannot be controlled with conservative treatment, he will develop hypertrophic synovitis. It is important to reach a differential diagnosis between hemarthrosis and synovitis. Acute hemarthrosis is associated with severe pain, and the joint is maintained in a position of comfort (typically in flexion). In contrast, chronic hypertrophic synovitis is associated with less pain. The synovium is palpable as a soft tissue firmness whereas a hemarthrosis will have a fluid characteristic.

Before making the recommendation of a synoviorthesis, the diagnosis should be confirmed by radiographs, echography, and/or magnetic resonance imaging (MRI). The differential diagnosis between synovitis and hemarthrosis can be determined by ultrasonography (echography) and MRI [8]. Radiographs should also be taken in order to assess the degree of hemophilic arthropathy at the time of diagnosis. In many situations, synovitis and hemarthrosis occur together. At the knee, echography is very specific and reliable.

Types of synoviorthesis

There are two basic types of synoviorthesis: chemical and radioactive. The materials most commonly used for chemical synovectomy are osmic acid, rifampicin, and oxytetracycline clorhidrate [9–12]. The radioisotopes currently used for radiation synovectomy are ^{90}Y (yttrium-90), ^{32}P (phosphorus-32), and ^{186}Rh (rhenium-186) [13–19]. Radiation synovectomy is the method of choice because it appears to be more efficacious than chemical synovectomy. Selection of the radioisotope should take into consideration the half-life, because the intensity of the inflammatory reaction is directly related to the rate of exposure and the size of the radiocolloid: the larger the size the less tendency for the material to leak from this joint space.

Table 28.1 Description of the radioisotopes most frequently used for radiosynoviorthesis in hemophilia.

	^{32}P	^{90}Y	^{186}Rh
Radioactive half-life (days)	14.3	2.8	3.8
Radiation	Beta	Beta	Beta and gamma
Therapeutic penetration power (mm)	2.2	2.8	1

Table 28.2 Clinical indications of the radioisotopes most frequently used for radiosynoviorthesis in hemophilia.

Isotope	Joint
^{90}Y and ^{32}P	Knees
^{186}Rh	Elbows, ankles

The material should be a pure β-emitting radioisotope, thereby minimizing the whole-body exposure to radiation that γ radiation can produce. ^{198}Au should probably not be used because it does emit γ radiation and is small in size [20]. Table 28.1 summarizes the main features of the radioisotopes most frequently used for radiosynoviorthesis in persons with hemophilia. Table 28.2 shows the clinical indications of the radioactive isotopes most frequently used for radiosynoviorthesis in hemophilia patients. The recommended isotope for the knee is ^{90}Y at a dose of 185 megabecquerels (MBq). ^{186}Rh is better for elbows (56–74 MBq) and ankles (74 MBq) [21].

The current measurement unit for radioisotopes is the becquerel (Bq), and the megabecquerel (MBq). Nowadays, however, it is common to encounter the former measurement unit, the millicurie (mCi), in many papers. According to Coya-Viña *et al*., the formula for the conversion of mCi into MBq is the following: 1 mCi = 37 MBq, and 1 mCi = 3.7×10^{10} disintegrations per second [21].

Taking into account the concern that radioactive materials evoke, and the high cost and limited supply of these materials, it is best to schedule groups of six to eight patients to perform radiation synovectomy. This will require some patients to wait upwards of 2–3 months until the whole group is scheduled for the procedure. If possible, patients should be maintained on continuous prophylaxis while awaiting the procedure. The aforementioned disadvantages of radiation synovectomy do not exist with rifampicin and oxytetracycline, which are very cheap and easily obtainable. Rifampicin injection can be rather painful and may have to be repeated at weekly intervals. The follow-up of oxytetracycline synoviorthesis is still very short (6 months) [12].

Age to perform synoviorthesis

Synoviorthesis can be performed at any age in the life of the hemophilic patient. Intra-articular injection in a very young child may be difficult because it requires patient cooperation and this may require general anesthesia. The potential of radiation-induced cellular damage or chromosomal abnormalities remain concerns, particularly in children. After more than 30 years of experience in using radiation synovectomy, neither articular nor systemic (neoplastic) damage has been reported. Regarding the maximum age for a synoviorthesis, it should be emphasized that the main consideration is that it should be performed for the correct indications, e.g., for recurrent hemarthrosis secondary to chronic synovitis. It should not be used for chronic effusions and pain secondary to degenerative disease (chronic hemophilic arthropathy).

The technique of synoviorthesis

If the procedure is performed under local anesthetic, the proposed site injection is infiltrated with up to 10 mL 1% lidocaine attempting to anesthetize not only the skin, but also the deeper tissues down to and including the joint capsule and synovium. For simple joint injections a 16- or 18-gauge spinal needle is sufficient but a wider bore 12- or 14-gauge needle (Abbocath or equivalent) may be necessary in order to evacuate a viscous hemarthrosis prior to joint injection. Once the joint has been entered, all the liquid content (blood or synovial fluid) should be evacuated, and only then should the fibrosing agent be injected. Postinjection pain may be reduced by mixing the agent with a long-acting local anesthetic, e.g., bupivacaine.

Chemical synovectomy requires no additional special precautions. However, when performing a radioactive synovectomy the needle should be withdrawn very slowly whilst at the same time injecting an anti-inflammatory drug (e.g., hydrocortisone acetate, triamcinolone) in order to avoid the risk of radioactive burn of the needle track or, even worse, an adjacent skin burn.

Whichever agent is employed, care must be taken to avoid extra-articular complications (radiation burn and/or inflammatory reaction) as a result of extravasation or needle track contamination. The most frequently affected joints in hemophilia are the elbows, the knees (Figure 28.1), and the ankles (Figure 28.2) [22].

Efficacy of synoviorthesis

On average, synoviorthesis has a 75–80% satisfactory outcome in the long term [2–7]. From the clinical standpoint, such efficacy can be measured by the decrease in the number of hemarthroses, with complete cessation for several years in some cases. In 20–25% of cases, synoviorthesis fails to control hemarthroses, but it can be repeated.

It is commonly accepted that the more severe the degree of synovitis, the more difficult it will be to remove the synovium by means of a synoviorthesis. In fact, in cases where marked

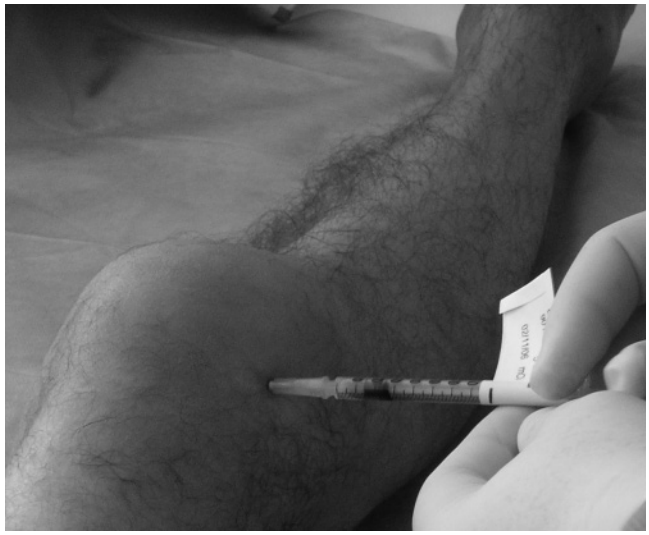

Figure 28.1 Knee synoviorthesis through the suprapatellar lateral route. The injection is made above the lateral corner of the patella and directly into the suprapatellar pouch.

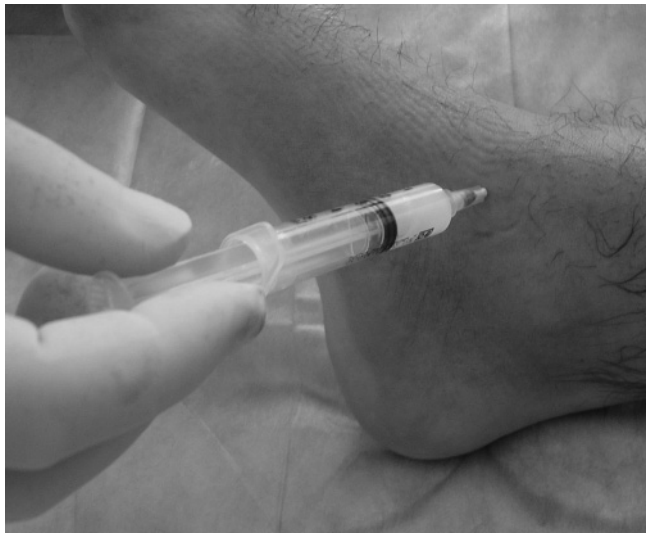

Figure 28.2 Ankle synoviorthesis through the anteromedial approach.

hypertrophy is present, it may be necessary to perform multiple consecutive synoviortheses, or even a surgical synovectomy. Synoviorthesis with rifampicin and oxytetracycline clorhydrate can be repeated many times at weekly intervals. With radiation synovectomy, no more than three synoviortheses are advised at 3-month intervals. When repeated synoviorthesis fail, a surgical synovectomy (open or arthroscopic) may be indicated.

Complications of synoviorthesis

The main complication of radiation synovectomy is a cutaneous burn if the radioactive material is injected out of the joint. Another potential complication is an inflammatory reaction after injection; in such a case, rest and nonsteroidal anti-inflammatory drugs (NSAIDs) will control these symptoms. No malignant effects (carcinogenic) of radioactive materials have been reported after more than 30 years of their use worldwide [23].

Multiple synoviorthesis in a single session

It is well known that the individual with hemophilia commonly has more than one target joint. In such cases, it is possible to perform more than one synoviorthesis in the same session. Two injections can be performed at the same time, and it is impractical to inject the same joint bilaterally, e.g., both elbows or both knees, both ankles, or two different joints of the lower limbs of different sides (e.g., right knee and left ankle). If two joints are to be injected, the two joints should be on the same side, e.g., elbow and knee, elbow and ankle, knee and ankle. Figure 28.3 summarizes the recommended treatment algorithm for synovitis and recurrent hemarthrosis in hemophilia with synoviorthesis.

Alternatives to synoviorthesis

A study was reported to determine optimal treatment for chronic hemophilic synovitis of the knee and synovitis of the elbow [19]. This included 65 patients with synovitis affecting 65 knee joints and 40 patients who had synovitis of the elbow (44 elbows), who, despite a 3-month trial of prophylactic substitution therapy, were treated by synovectomy during 1974 to 1996. Radiation synovectomies (^{198}Au synoviorthesis) were performed on 38 knees, open surgical synovectomy on 18, and nine had an arthroscopic procedure. Radioactive gold synoviorthesis was performed on 29 elbows, and 15 had a resection of the radial head and partial open synovectomy. This study concluded that synovectomy (by any method) significantly reduced bleeding episodes, but did not halt the radiographic deterioration of the joints.

It is thought that radiation synovectomy is the best choice for patients with persistent synovitis of the knee and elbow unresponsive to a 3-month trial of prophylactic factor replacement (Table 28.3). If two to three consecutive synoviortheses given at 3-month intervals fail, a surgical synovectomy is indicated (Figure 28.3). When chronic synovitis is allowed to persist, the membrane can hypertrophy to the point where it cannot be adequately ablated by a pure β-emitting radiocolloid which penetrates only between 2.2 mm and 2.8 mm (Table 28.1). In these cases, as well as those in which repeated radiosynoviorthesis has failed, surgical synovectomy (open or arthroscopic) is often effective [7].

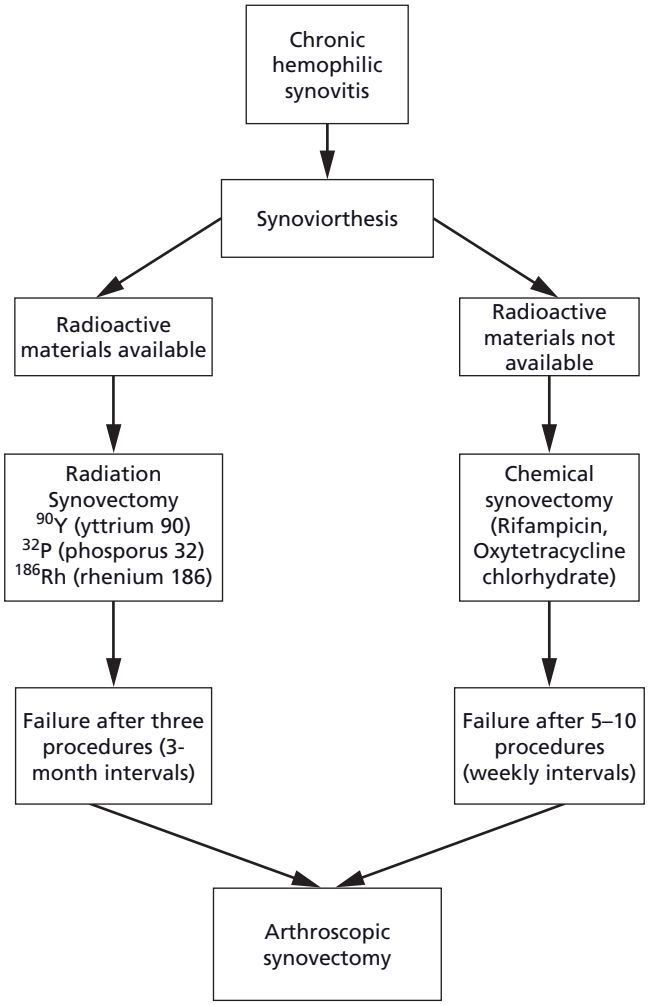

Figure 28.3 Treatment algorithm recommended by the author when treating a patient with chronic hemophilic synovitis. Adapted from [3] with permission.

Table 28.3 Synoviorthesis versus synovectomy.

Synoviorthesis	Surgical synovectomy
Inexpensive	Expensive
Simple	Complex
Painless	Painful
Less invasive	Invasive
Lower risk of infection	High risk of infection
No general anesthetic	General anesthetic

Radiosynoviorthesis is necessary in hemophilic patients despite prophylaxis

Hematologic prophylaxis currently is the most recommended method to control the articular bleeding of hemophilia. We performed a study to determine how many patients with severe hemophilia on prophylaxis required radiosynoviortesis (RS) and to compare them with those with mild or moderate hemophilia treated on demand [24].

We retrospectively reviewed 93 patients with severe hemophilia treated on prophylaxis (group P) and 106 patients with mild and moderate hemophilia treated on demand (group D), between 2003 and 2005. The average age of patients in both groups was similar. In group P there were 79 with hemophilia A and 14 with hemophilia B. They were treated with 50 IU/kg three days a week in hemophilia A and two days a week in hemophilia B. In group D, 39 patients had moderate hemophilia (29 type A and 10 type B) and 67 had mild hemophilia (63 type A and 4 type B).

Eleven patients in group P required RS (11.82%). All of them had severe hemophilia A. Three of the 11 patients needed RS in more than one joint. Fourteen joints were treated overall (four elbows, five knees, and five ankles). Four patients in group D required RS (3.77%). All of them were moderate hemophiliacs, three type A and one type B. The RS were performed in three knees and one elbow.

We observed that prophylaxis in hemophilic patients does not avoid the development of articular complications. In our series 11.82% required RS. All of them had severe hemophilia A. There were no differences between joints (elbow, knee, and ankle). Patients with severe hemophilia on prophylaxis required more RS (12%) than patients with mild or moderate hemophilia treated on demand (3.77%) [24].

Which type of synoviorthesis is better?

It is important to emphasize that controversy exists regarding which type of synoviorthesis is better. Most authors in developed countries use radiosynoviorthesis (^{90}Y, ^{32}P, ^{186}Rh), whereas others utilize chemical synoviorthesis (rifampicin, oxytetracycline) mainly because of the lack of availability of radioactive materials. The general recommendation is to use radiosynoviorthesis because it has proved to be efficient for the treatment of chronic hemophilic synovitis, even in patients with inhibitors. Moreover, no complications related to the use of radioactive materials have been reported after more than 30 years of being used worldwide.

Comparative studies in different joints, with different materials, ^{90}Y and ^{186}Rh are required to clarify which synoviorthesis is best.

We must design scientific research on synoviorthesis taking into account not only the radioactive material to be used, but also the different joints (elbows, knees, and ankles). Needless to say that the age of the patients, the degree of synovitis, the different doses, and many more variables are very difficult to control. Synoviorthesis (irrespective of the age of the patient), should be performed early, that is to say, as soon as synovitis is diagnosed. ^{186}Rh is recommended for the elbows and ankles, and ^{90}Y for the knees; both radioactive materials appear to be better than ^{32}P. In North America ^{32}P is used because it is the

isotope approved by the Food and Drug Administration. There is no doubt that radiosynoviorthesis is an excellent, cheap, easy, and safe procedure for the treatment of hemophilic synovitis.

Conclusion

Synoviorthesis is a very effective procedure that decreases both the frequency and the intensity of recurrent intra-articular bleeds related to joint synovitis. Radiosynoviorthesis is currently recommended with ^{90}Y for the knees and ^{186}Rh for elbows and ankles. The procedure should be performed as soon as possible to minimize the degree of articular cartilage damage. It can also be used in patients with inhibitors with minimal risk of complications [25,26].

On average, synoviorthesis has a 75–80% satisfactory outcome in the long term. Such efficacy can be measured clinically by the decrease in the number of hemarthroses, with complete cessation for several years in some cases. In 20–25% of cases, synoviorthesis fails to control hemarthroses, but in such cases, it can be repeated [27]. Global long-term results of treatment with chemical synovectomy (rifampicin and oxytetracycline) seem to be less favorable than with radionuclides (^{90}Y, ^{32}P, and ^{186}Rh).

References

1 Molho P, Verrier P, Stieltjes N, *et al*. A retrospective study on chemical and radioactive synovectomy in severe haemophilia patients with recurrent haemarthrosis. *Haemophilia* 1999; **5**: 115–23.

2 Salis G, Molho P, Verrier P, *et al*. Nonsurgical synovectomy in the treatment of arthropathy in Von Willebrand's Disease. *Rev Rhum Engl Ed* 1998; **65**: 232–7.

3 Rodriguez-Merchan EC, Wiedel JD. General principles and indications of synoviorthesis (medical synovectomy) in haemophilia. *Haemophilia* 2001; **7**(Suppl. 2): 6–10.

4 Rodriguez-Merchan EC, Goddard NJ. Chronic haemophilic synovitis. In: Rodriguez-Merchan EC, Goddard NJ, Lee CA (eds.) *Musculoskeletal Aspects of Haemophilia*. Oxford: Blackwell Science Ltd, 2000: 43–9.

5 Fernandez-Palazzi F. Treatment of acute and chronic synovitis by non-surgical means. *Haemophilia* 1998; **4**: 518–23.

6 Fernandez-Palazzi F, Rivas S, Cibeira JL, Dib O, Viso R. Radioactive synoviorthesis in hemophilic hemarthrosis: Materials, techniques, and dangers. *Clin Orthop Relat Res* 1996; **328**: 14–18.

7 Rodriguez-Merchan EC, Luck JV, Jr., Silva M, Quintana M. Synoviorthesis in haemophilia. In: Rodriguez-Merchan EC (ed.) *The Haemophilic Joints. New Perspectives*. Oxford: Blackwell Science Ltd, 2003: 73–9.

8 Nuss R, Kilcoyne RF, Rivard GE, Murphy J. Late clinical, plain X-ray and magnetic resonance imaging findings in haemophilic joints treated with radiosynoviorthesis. *Haemophilia* 2000; **6**: 658–63.

9 Caviglia HA, Fernandez-Palazzi F, Maffei E, *et al*. Chemical synoviorthesis for hemophilic synovitis. *Clin Orthop Relat Res* 1997; **343**: 30–6.

10 Caviglia H, Galatro G, Duhalde C, Perez-Bianco R. Haemophilic synovitis: Is rifampicin an alternative? *Haemophilia* 1998; **4**: 514–17.

11 Fernandez-Palazzi F, Rivas S, Viso R, de Bosch NB, de Saez AR, Boadas A. Synovectomy with rifampicin in haemophilic haemarthrosis. *Haemophilia* 2000; **6**: 562–5.

12 Fernandez-Palazzi F, Viso R, Bernal R, Capetillo G, Caviglia H. Oxytetracycline clorhydrate as a new material for chemical sinoviortesis in haemophilia. In: Rodriguez-Merchan EC (ed.) *The Haemophilic Joints. New Perspectives*. Oxford: Blackwell Science Ltd, 2003: 80–3.

13 Heim M, Horoszowski H, Lieberman L, *et al*. Methods and results of radionuclide synovectomies. In: Gilbert MS, Greene WD (eds.) *Musculoskeletal Problems in Hemophilia*. New York: National Hemophilia Foundation, 1990: 98–101.

14 Rodriguez-Merchan EC. Methods to treat chronic haemophilic synovitis. *Haemophilia* 2001; **7**: 1–5.

15 Mathew P, Talbut DC, Frogameni A, *et al*. Isotopic synovectomy with P-32 in paediatric patients with haemophilia. *Haemophilia* 2000; **6**: 547–55.

16 Heim M, Goshen E, Amit Y, Martinowitz U. Sinoviortesis with radioactive Yttrium in haemophilia: Israel experience. *Haemophilia* 2001; **7**(Suppl. 2): 36–9.

17 Rodriguez-Merchan EC, Jimenez-Yuste V, Villar A, Quintana M, Lopez-Cabarcos C, Hernandez-Navarro F. Yttrium-90 synoviorthesis for chronic haemophilic synovitis: Madrid experience. *Haemophilia* 2001; **7**(Suppl. 2): 34–5.

18 Silva M, Luck JV, Jr., Siegel ME. ^{32}P chromic phosphate radiosynovectomy for chronic haemophilic synovitis. *Haemophilia* 2001; **7**(Suppl. 2): 40–9.

19 Rodriguez-Merchan EC, Magallon M, Galindo E, Lopez-Cabarcos C. Hemophilic synovitis of the knee and the elbow. *Clin Orthop Relat Res* 1997; **343**: 47–53.

20 Rodriguez-Merchan EC, Magallon M, Martin-Villar J, *et al*. Long term follow up of haemophilic arthropathy treated by ^{198}Au radiation synovectomy. *Int Orthop* 1993; **17**: 120–4.

21 Coya-Viña J, Marin-Ferrer M, Martin-Curto LM. Radioactive isotopes for radiosynoviorthesis. In: Rodríguez-Merchan EC (ed.) *The Haemophilic Joints. New Perspectives*. Oxford: Blackwell Science Ltd, 2003: 68–72.

22 Rodriguez-Merchan EC, Goddard NJ. The technique of synoviorthesis. *Haemophilia* 2001; **7**(Suppl. 2): 11–15.

23 Falcon de Vargas A, Fernandez-Palazzi F. Cytogenetic studies in patients with hemophilic hemarthrosis treated by ^{198}Au, ^{186}Rh, and ^{90}Y radioactive synoviorthesis. *J Pediatr Orthop B* 2000; **9**: 52–4.

24 Rodriguez-Merchan EC, de la Corte-Rodriguez H, Romero-Garrido JA, *et al*. Radiosynoviorthesis is necessary in haemophilic patients despite prophylaxis. *J Thromb Haemost* 2007; **5**(Suppl. 2): P-W-126.

25 Lofqvist T, Petersson C, Nilsson IM. Radioactive synoviorthesis in patients with hemophilia with factor inhibitor. *Clin Orthop Relat Res* 1997; **343**: 37–41.

26 Petersson CJ, Berntorp E. Radiosynoviorthesis in patients with haemophilia and inhibitors. In: Rodriguez-Merchan EC, Lee CA (eds.) *Inhibitors in Patients with Haemophilia*. Oxford: Blackwell Science Ltd, 2002: 129–31.

27 Rodriguez-Merchan EC. Radionuclide synovectomy (radiosynoviorthesis) in hemophilia: A very efficient and single procedure. *Sem Thromb Hemost* 2003; **29**: 97–100.

29 Pseudotumors in patients with hemophilia

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Introduction

Pseudotumor, an expending destructive encapsulated cyst/hematoma, is a rare but serious complication in persons with hemophilia (PWH). Its estimated incidence is reported at about 1%.

To date, only anecdotal reports and a few small case series have been published in the literature. Historically, these reports originated mainly from countries with limited resources where replacement therapy was not adequate. An assumption was thus made that there is a connection between the lack of factor replacement therapy and the development of pseudotumors. This suspicion has been strengthened by the reports of the occurrence of pseudotumors in patients who have developed an antibody to the missing coagulation factor. There is no established standard approach today to the management of pseudotumors and the treatment is based on clinical rationale and the opinion of experts with limited experience.

Pathogenesis of pseudotumors

There are two distinct pathologic forms that have been noted and categorized under the heading of pseudotumors. The first form occurs within the peripheral long bones and very often in the developing skeleton, while the second has a predilection particularly for the area of and around the pelvis. The former starts as an intraosseous expansion that can perforate the cortex, while the latter begins its growth in the soft tissues and may erode skeletal structures. Some authors believe that the pathology starts in the Sharpley's fibers (the tissue connecting muscles to the periosteum).

Anatomically, this situation exists extensively around the pelvis and the thighs [1]. The intraosseous form is more aggressive then a simple bone cyst, for it actively expands, and yet is amenable to conservative treatment [2]. The soft-tissue masses have a distinct histologic structure [3]. The contents comprise necrotic blood but may also contain large quantities of blood and other liquified tissue products. The pseudotumor has a thick capsule wherein blood vessels are encased and infuse the entire pseudotumor. The mass is expansive and hence has the ability to cause pressure necrosis of the surrounding tissues, which can include the cortex of bone (Figures 29.1, 29.2, and 29.3 and Plate 29.1). The pseudotumor may expand around structures such as the ureters, blood vessels, and nerves. These structures are not invaded by the tumoral growth but encircled, and upon gross anatomy would appear to be within the tumor mass [4].

Clinical presentation

As stated previously, there are two forms of pseudotumors.

The peripheral pathology is generally amenable to early diagnosis in view of the anatomical deformation of fingers and toes and/or the perceivable swelling noted over the dorsum of the hand or foot. Radiographic evidence is easily obtained and treatment can then be instituted.

The second form is usually intra-abdominal and slow growing. Early diagnosis rarely occurs as the expanding mass slowly fills up the area of the abdomen and/or the pelvis and retroperitoneum. The patients may note a general discomfort, but in the light of their experiences with intra-articular hemarthroses and muscle bleeds these generalized symptoms are often ignored. Radiologic plates may show a displacement of viscera and/or bone erosion and a more extensive investigation is then indicated in order to assess the extent of the pseudotumor.

Investigations prior to treatment

Although, as stated earlier, usually arising in the area of the pelvis, large pseudotumors can theoretically appear anywhere in the body. It must be appreciated that not only are the dimensions of the mass relevant, but also the contents and the anatomy of the surrounding area. The following investigations should be considered:

- Radiologic assessment in order to ascertain the osseous extent of the damage—regular radiographs and computed tomography usually suffice.
- The assessment of the structure and nature of the soft tissues and their relations to the bone can be mapped out by ultrasound and magnetic resonance imaging (MRI). Intravenous injection of gadolinium in conjunction with the MRI is

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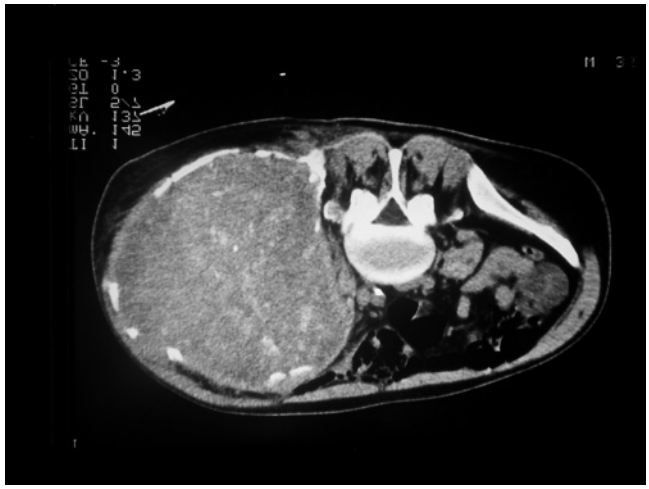

Figure 29.1 Computed tomography section through the level of the lumbar spine. A huge round pseudotumor can be noted. The pseudotumor has destroyed and fragmented the ileum and the mass extends almost to the midline.

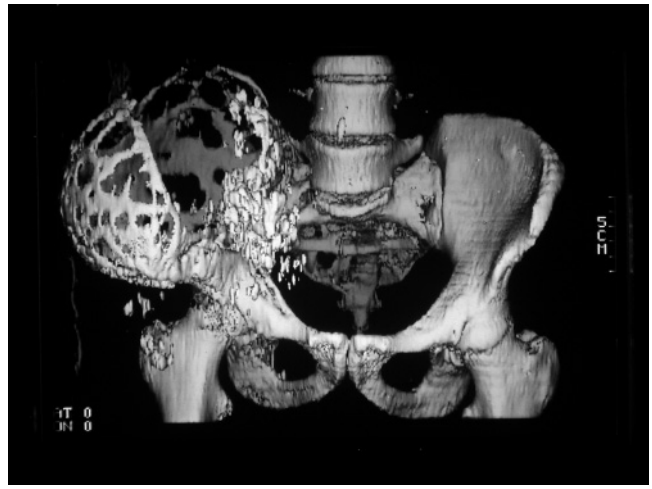

Figure 29.3 This figure clearly shows a computed tomography reconstruction of the pseudotumor, which has fragments of the ileum surrounding the mass. Note the encroachment onto the sacrum and the destroyed sacroiliac articulation.

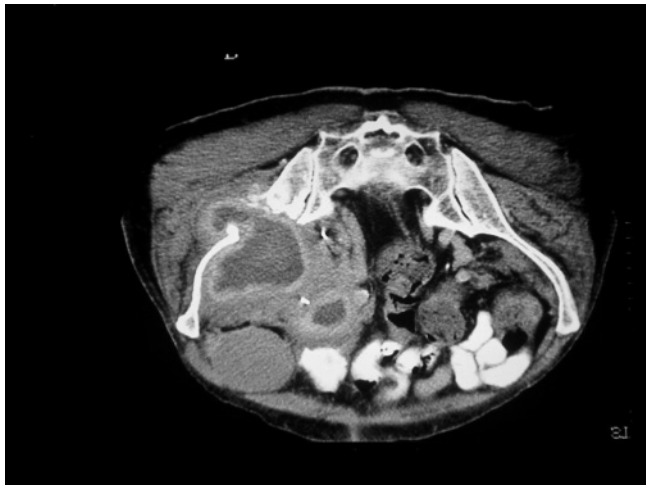

Figure 29.2 The computed tomography section shows a large pseudotumor mass that has expanded through the ileum. The mass can clearly be seen in the destroyed bone and on both sides of the ileum.

suggested. The gadolinium helps to detect pathologic blood vessels and also helps to delineate the pseudotumor capsule.

- Intravenous pyelography should be considered if the growth is in the region of the kidney and its draining apparatus.
- It is important to evaluate the anatomy of major blood vessels in or close to the mass on arteriogram to plan the best surgical approach.

The planning of the operation should comprise a multidisciplinary team of experts, including an expert in invasive radiology, orthopedic and vascular surgeons, a urologist if the pelvis is involved, and—if required—spine, liver, general neurosurgeons and any other specialist, depending on the expanse of the pseudotumor. It is of the utmost importance to appoint a coordinator (usually a surgeon, but could also be an experienced hemophilia expert) to coordinate the rotation of the various experts according to the progress of the surgery. Experts can be summoned a few times during the surgery, therefore the members of the multidisciplinary team should be informed to be available during the whole surgical procedure, which may take several hours.

Hematologic assessment of the patients is essential and should include inhibitor testing and full pharmacokinetic evaluation to find the individual recovery, half-life, and clearance of the patient's missing factor prior to the surgery.

There should be a coagulation team and laboratory service available during the surgery and postoperative care. Sufficient concentrates should be reserved for the surgery and postsurgical period. High trough levels should be maintained over a period of 10 days to 3 weeks. According to our limited experience with four giant pseudotumors (two in Israel and two abroad with our participation and guidance) [5] the levels drop below the planned trough owing to high consumption. All four patients had diffuse bleeding from the extensive tissue damage. Fibrin glue was sprayed over the surfaces to assist local hemostasis but was ineffective in two operations because of the active bleeding that washed away the glue before its clotting. rFVIIa immediately stopped the diffuse bleed and allowed the use of fibrin glue (to prevent a re-bleed.) The most appropriate approach is to deliver the replacement therapy by continuous infusion as described in the chapter on continuous infusion in the current book (Chapter 6). In the event of partial resection of the giant pseudotumor, an extended period of replacement treatment with higher trough levels may be necessary. Two of our four patients mentioned required trough levels of FVIII above 10% for periods of 6 months and

2 years, respectively. Any attempt to reduce the levels ended in retroperitoneal bleeding. One patient was living in another country (about an hour's flying time) and once a week he sent a plasma sample by plane. His factor VIII level was tested and in the evening he received instructions on the new rate of infusion required to maintain the desired level. The patient had a minipump and the necessary disposable sets and was trained to perform continuous home infusion [6].

Additional hemostatic measure

Owing to the extensive surgery, factor replacement alone may not be sufficient to control the diffuse and massive bleeding. The addition of fibrinolytic inhibitor is optional (high doses similar to cardiac surgery, as tranexamic acid 2 g every 4 h). The use of fibrin glue is recommended.

Prior to surgery

As stated previously, the pseudotumor receives its blood supply from vessels that are present within the capsule that surrounds the mass. These vessels have a number of "feeding" arteries and if they can be thrombosed the risk of massive intraoperative bleeding can be reduced [7] and the pseudotumor mass may be reduced in size. Arteries have the ability of recanalization and hence the thrombosis should be carried out 7–10 days before surgery.

Realistic aims

The intention should be the entire eradication of the pseudotumor and the reconstruction of the normal anatomy. This is not always possible as there may be destroyed bone. The displaced viscera are not usually a problem. Bony structures, e.g., the iliac bone, should be left in a stable condition [8], and if large bone fragments remain viable, but free floating after the tumor has been excised, it is suggested that fixation methods be implemented [9]. "Dead space" can be "plugged" by the insertion of the omentum [10]. Bone allografts and/or bone substitutes can be used together with fibrin glue in the reconstruction of skeletal integrity. The peripheral pseudotumors are easily treated by the opening of a bone window, the drainage and curettage of the contents and then the packing of the pseudotumor space with bone and/or bone substitutes [11] mixed with glue [12].

Total excision is the intention, but is not always possible. The dissection is carried out around the capsule of the pseudotumor, but, as stated previously, vital structures, although completely surrounded by the capsule, may be very deeply "embedded" within the contents of the pseudotumor. Structures such as the ureter can be damaged easily even though a catheter may have been inserted previously. It is probably safer to leave a small fragment of the pseudotumor wall attached to the vital structure than to risk damaging it. Abdominal pseudotumors may be enormous, and the authors have managed a case in which 6 L of liquid contents was drained out of the pseudotumor.

Complications

The complications may be divided into two groups, intra- and perioperative, and late complications. The former relates to the surgical procedure, which may require a multidisciplinary surgical team to conduct complicated surgery in a very protracted procedure. Infection is always a risk but even more so in the late recovery phase. The formation of a constantly draining sinus creates a situation wherein an open canal exists between the exterior, the bowel [13], and the abdominal cavity. This situation inevitably results in a purulent discharge and may lead to death. Another complication is the regrowth or regeneration of a pseudotumor. As stated previously, intra-abdominal pseudotumors are usually only diagnosed at a late stage, when huge expansion and destruction has taken place. If the pseudotumor is recognized early, excision can be less complicated. Excision causes extensive alteration in the soft-tissue anatomy owing to the extensive healing fibrosis and adhesions. Postexcision follow-up is necessary in order to document and discover whether the pseudotumor has re-formed and started growing once again. The use of radiotherapy for the management of pseudotumors is not needed. Hilgartner *et al.* [14] reported using this modality in 1975. Espandar *et al.* [15] retested the use of radiotherapy suggesting doses between 6 and 23.5 Gy by delivery in small fragments. It was postulated [14] that the radiotherapy affects the feeding blood vessels to the growth and hence its curative effect. Kapoor *et al.* [16] questioned and reviewed the results of radiotherapy treatment. Post-therapy recurrences of the pseudotumors were reported. From the analysis of the literature review, it appears that the results of treatment with radiotherapy and factor replacement versus radiotherapy alone produce similar results. Gilbert [17] described two types of pseudotumors: proximal and distal. The proximal involve the pelvis and long bones while the distal are in the small bones of the hands and feet. The latter group has been successfully managed by factor replacement only.

It should be stressed and noted that the hematologic control of the coagulation status of these patients may be complicated by acute hemorrhaging (requiring massive blood replacement), large oozing areas, and the fact that the patient may have an inhibitor to the missing coagulation factor. Factor assays should be carried out during the preoperative period and ample supplies of relevant clotting factor should be present and not just on standby elsewhere.

The physical rehabilitation of patients after such massive surgery requires the dedication of the rehabilitation team. Initially, the patient requires intensive nursing care. The wound dressing and the general nursing guides the patient

from the postoperative catabolic state into an anabolic phase. Psychological support is essential for the patient and the family. Progress is slow and the physical therapist and the occupational therapist enhance joint movements and muscle strengthening. Once the wounds have healed, hydrotherapy is added to the therapeutic regime and more intensive physical therapy. During this rehabilitation period, which starts initially on an inpatient basis and progresses to an ambulatory service, the authors have found it necessary from time to time to remind the patient of his status quo prior to the surgery. This reinforcement assists the patient in appreciating the improvement in the quality of life.

In conclusion, where surgery is necessary it should be carried out in a tertiary medical center by the most experienced multidisciplinary team. For more information, a short list of the key references has been provided. There are many case reports in the older medical literature that have been omitted from this list. The major issues are covered by the references.

References

1 Duthie R, Matthews J, Rizza C, Steel W. Haemophilic cysts and pseudotumours. In: Duthie R, Matthews J, Rizza C, Steel W (eds.) *The Management of Musculoskeletal Problems in the Haemophilias*. Oxford: Blackwell Scientification Publications, 1972: 84–98.

2 Gilbert MS, Forster A. A rational approach to the treatment of haemophilic blood cysts (pseudotumours) in patients with inhibitors. In: Rodrigues Merchan EC, Lee CA (eds.) *Inhibitors in Patients with Haemophilia*. Oxford: Blackwell Science, 2002: 142–45.

3 Rodrigues Merchan EC. The haemophilia pseudotumour. *Haemophilia* 2002; **8**: 12–16.

4 Heim M, Luboshitz J, Amit Y, Martinowitz U. The management of giant haemophilic pseudotumours. In: Rodrigues Merchan EC, Goddard NJ, Lee CA (eds.) *Musculoskeletal Aspects of Haemophilia*. Oxford: Blackwell Science, 2000: 105–11.

5 Valentino LA, Martinowitz U, Doolas A, Murali P. Surgical excision of a giant pelvic pseudotumour in a patient with haemophilia A. *Haemophilia* 2006; **12**: 541–4.

6 Martinowitz U, Schulman S, Gitel S, *et al*. Continuous infusion of factor concentrates in children. In: *Handbook of Hemophilia*. New York: American Elsevier Publishing, 1975: 569–89.

7 Sevilla J, Alvarez MT, Hernandez D, *et al*. Therapeutic embolization and surgical excision of haemophilic pseudotumour. *Haemophilia* 1999; **5**: 360–63.

8 Heeg M, Van Smit M, de Meer J, Van Horn JR. Excision of a haemophilic pseudotumour of the ileum complicated by fistulation. *Haemophilia* 1998; **4**: 132–5.

9 Ishiguro N, Iwahosi Y, Kato T, *et al*. The surgical treatment of haemophilic pseudotumours on an extremity: a report of three cases with pathological fractures. *Haemophilia* 1998; **4**: 126–31.

10 Bellinazzo P, Silvello L, Caimi T, *et al*. Long-term evaluation of a novel surgical approach to the pseudotumour of the ileum in haemophilia: Exeresis and transposition of the omentum in the residual cavity. *Haemophilia* 2000; **6**: 702–4.

11 Sagarra M, Lucas M, de La Torre G, *et al*. Successful surgical treatment of haemophilic pseudotumour, filling the defect with hydroxyapatite. *Haemophilia* 2000; **6**: 55–6.

12 Caviglia HA, Fernandez-Palazzi F, Galatro G, *et al*. Percutaneous treatment of haemophilic pseudotumours. In: Rodrigues-Merchan EC, Goddard NJ, Lee CA (eds.) *Musculoskeletal Aspects of Haemophilia*. Oxford: Blackwell Science, 2000: 97–104.

13 Heaton D, Robertson R, Rothwell A. Iliopsoas haemophilic pseudotumour with bowel fistulation. *Haemophilia* 2000; **6**: 41–3.

14 Hilgartner M, Arnold W. Hemophilic pseudotumor treated with replacement therapy and radiation. *JBJS* 1975; **57A**: 1145–46.

15 Espandar R, Heidaic P, Rodriguez-Merchan EC. Management of haemophilic pseudotumours with special earplysis on radiotherapy and arterial embolization. *Haemophilia* 2009; **15**: 448–57.

16 Kappor R, Sastric J, Malhotra P, Kumar V, Singh P. Hemophilic pseudotumour—is there a role of radiotherapy? A case report with review of the literature. *Turk J Hematol* 2006; **23**: 53–8.

17 Gilberg MS. Characterizing the hemophilic pseudotumor. *Ann NY Acad Sci* 1975; **240**: 313–15.

30 Imaging modalities for assessment of hemophilic arthropathy*

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Introduction

The diagnosis of hemophilia is based on clinical data and laboratory tests, and radiologic imaging is used to evaluate complications of hemophilia. Recurrent joint bleeds resulting in progressive arthropathy constitute the main cause of morbidity in persons with hemophilia. It is important to have objective means of diagnosing, assessing, and monitoring joint changes in order to make therapeutic decisions in clinical work and also to allow scientific comparisons of the efficiency of different therapeutic regimens. Technical innovations in the field of radiology add new imaging modalities to the diagnostic armamentarium. This chapter discusses conventional radiography (X-ray), magnetic resonance imaging (MRI), and ultrasonography, as well as some novel imaging techniques that may become useful for evaluating hemophilic arthropathy.

Radiography (X-ray)

X-rays have been used in diagnostic imaging for more than a century. The scattering of X-rays penetrating the object of interest is the basis of image contrast, which can differentiate metal, bone, soft tissues (equals water), fat, and gases [1]. In hemophilia, X-rays are used to evaluate changes in joints. Even though newer imaging modalities have been introduced, X-ray examination still represents the gold standard for structural assessment of hemophilic arthropathy. Furthermore, the widespread availability and long experience of X-ray methods guarantees that, in a global perspective, this technology will continue to be an important tool in the foreseeable future.

Most of the radiographic findings in hemophilic joints appear in a given sequence and are essentially the same in all joints, although some changes are characteristic of certain types of joints [2–5].

In any joint, a hemarthrosis can cause dissention of the joint capsule, and repeated bleeds will lead to synovial hypertrophy, effusion, and periarticular edema, which are changes that can appear as nonspecific soft tissue swelling in the X-ray images. Hemosiderin deposits develop and can occasionally be seen as dense areas in the images. Increased perfusion secondary to the inflammatory reaction results in accelerated bone maturation in children, and it also causes overgrowth of the epiphyses and periarticular osteopenia in both children and adults. Moreover, osteopenia can be aggravated by disuse. Irregularity of the subchondral bone arises, and progressing cartilage destruction leads to joint space narrowing. Marginal erosions, subchondral cysts, and destruction of subchondral bone may also occur (Figure 30.1). Eventually, without treatment, an end stage is reached that entails severe deformity and fibrous or bony ankylosis.

Ankles, knees, and elbows are the joints most often involved. Characteristic in the ankle is deformation of the talus accompanied by valgus deformity secondary to narrowing of the lateral part and widening of the medial part of the tibial epiphysis. Typical changes in the knee are squaring of the patella and overgrowth of the femoral condyles with widening of both the intercondylar notch and the groove between the tibial spines. In the elbow, specific findings include enlargement of the radial head and subsequent increase in the size of the radial notch of the ulna, as well as enlargement of the distal humerus along with widening of the olecranon fossa, which causes thinning of the bone wall, or even the formation of an olecranon foramen.

The hips, shoulders, and wrists can also be affected by arthropathic changes, albeit less often, and involvement of other types of joints is rare. In the hip, vascular necrosis can lead to enlargement or resorption of the femoral head and in late stage thinning of the medial wall of the acetabulum, and

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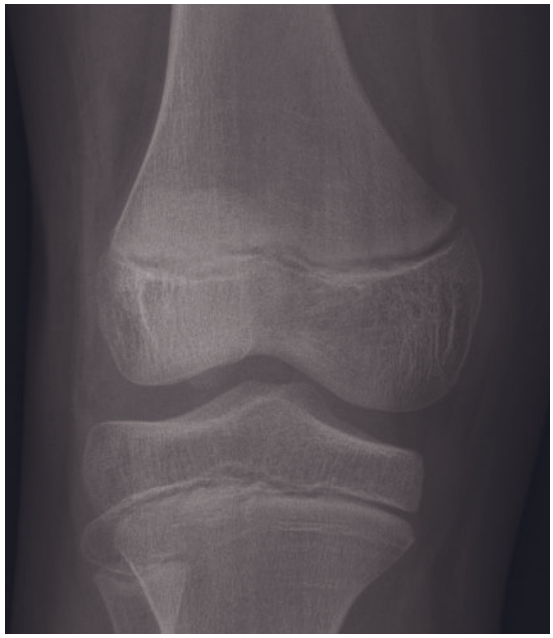
*Bjorn Lundin is author of the two parts "MRI" and "Radiography", and Andrea Doria is author of the two parts "Ultrasonography" and "Novel imahing techiques" (including both new MRI and Ultrasonograpphy techniques)



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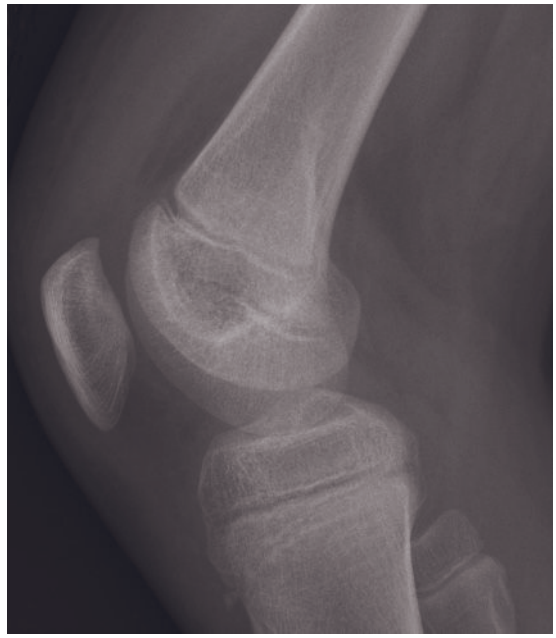

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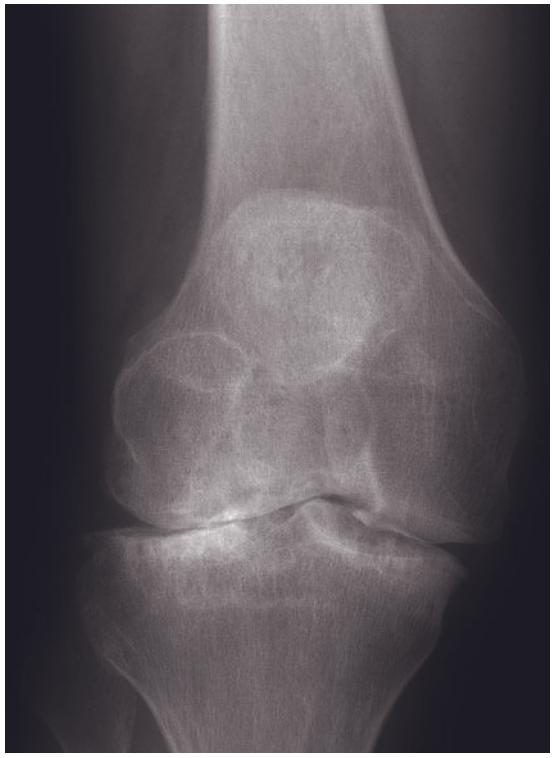

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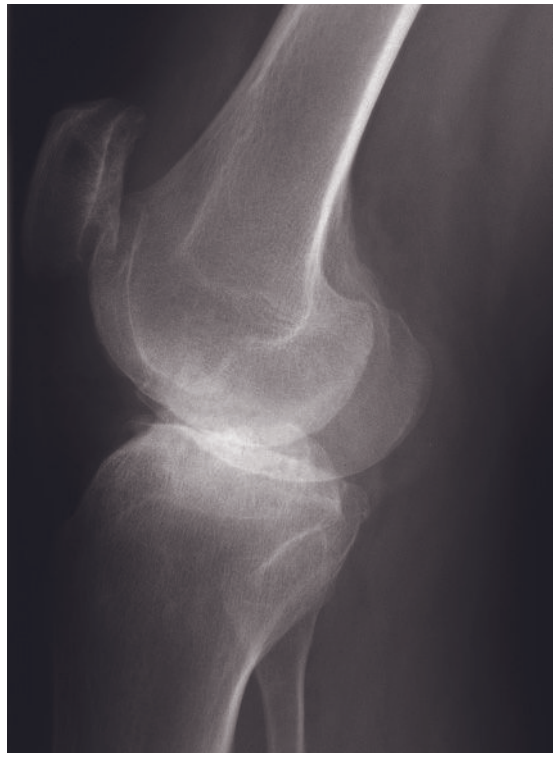


(d)

acetabular protrusion, can appear. In the shoulder, first enlargement and later atrophy of the humeral head may occur, and, in the wrist, luxation of the distal radioulnar joint secondary to growth disturbances can arise. Before the advent of modern treatment, there were also rare reports on involvement of the metacarpophalangeal joints of the hand, as well as changes in the posterior subtalar joints and enlargement of the sinus tarsi of the foot.

X-ray is used for planning and as a baseline examination before therapeutic measures such as arthrodesis and joint replacement. Importantly, X-ray imaging is also employed to follow the progression of an arthropathy as a means of monitoring the effects of clinical therapy and to achieve research evaluation of treatment efficacy. The Arnold–Hilgartner scale [2] and the Pettersson score [6] are the two widely used radiographic classification systems for hemophilic arthropathy, and the World Federation of Hemophilia recommends the use of the latter system. Over the past decades, radiographic scoring has greatly aided appraisals of hemophilia care [7,8]. However, X-ray imaging is limited in that it cannot detect the earliest arthropathic changes, and countries with well-developed healthcare systems are now in need of more sensitive methods for analyzing the therapeutic efficiency and cost benefits of new intense factor prophylactic regimens for hemophiliacs [9].

Magnetic resonance imaging

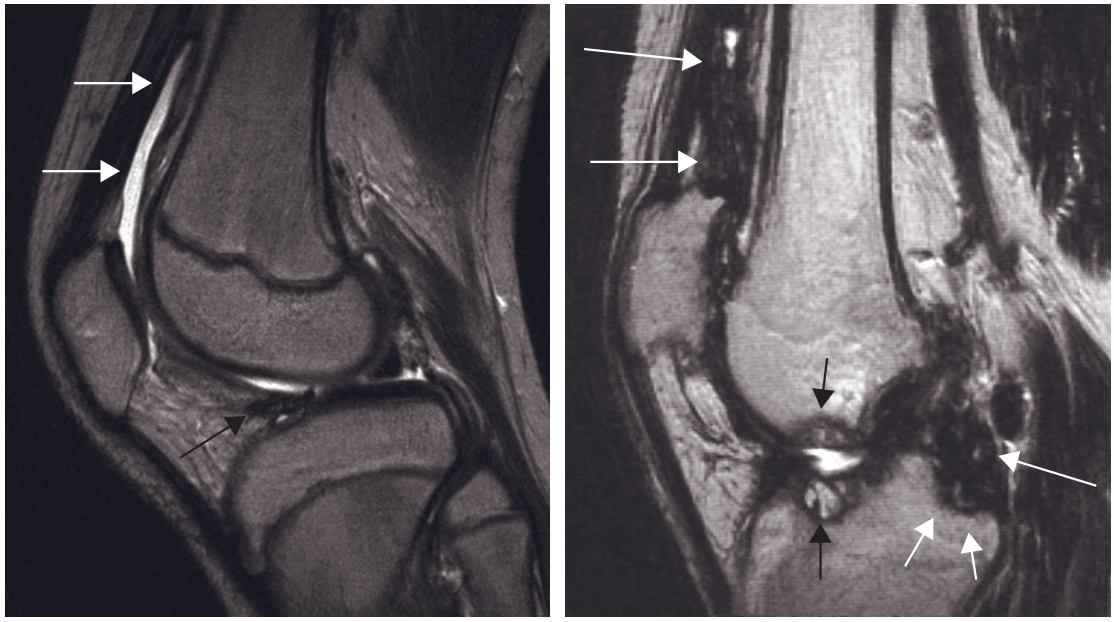
Magnetic resonance imaging (MRI) was introduced as a medical imaging modality in the 1980s, and, instead of being based on ionizing radiation as in X-ray imaging, it uses radiowaves and magnetic fields [10]. MRI provides images with high soft-tissue contrast in any plane, which is extremely valuable when evaluating musculoskeletal disorders [1]. In hemophilia, MRI can be used to assess pseudotumors and intracranial, intraspinal, and intramuscular bleeds, as well as articular complications, but this chapter is focused on changes in joints. MRI has obvious advantages, and hence, when available, it should be the preferred modality in several situations. However, it should also be noted that MRI is a more advanced and costly technique and that in many settings it is less accessible than X-ray imaging. In addition, the task of imaging is time consuming, which makes it difficult to investigate multiple joints.

A gradient echo T2*-weighted sequence is often used in MRI analysis of joints in hemophiliacs. This sequence can visualize many of the changes that are of interest in hemophilic arthropathy, and it allows especially sensitive detection of hemosiderin owing to the artifactual signal loss caused by iron. Survey imaging with only a T2*-weighted gradient echo sequence is relatively fast and represents an option that can enable multiple joint investigations. Single joints are usually evaluated by imaging protocols that include combinations of images with different weightings and dedicated cartilage sequences, and that type of strategy reveals some types of lesions more efficiently, but is more time consuming and therefore less practical for multiple joints [11–19]. The use of gadolinium contrast agent is sometimes warranted in other inflammatory arthritis, such as rheumatoid arthritis, but so far investigators have not been able to confirm similar advantages of this contrast agent in the evaluation of hemophilic arthropathy.

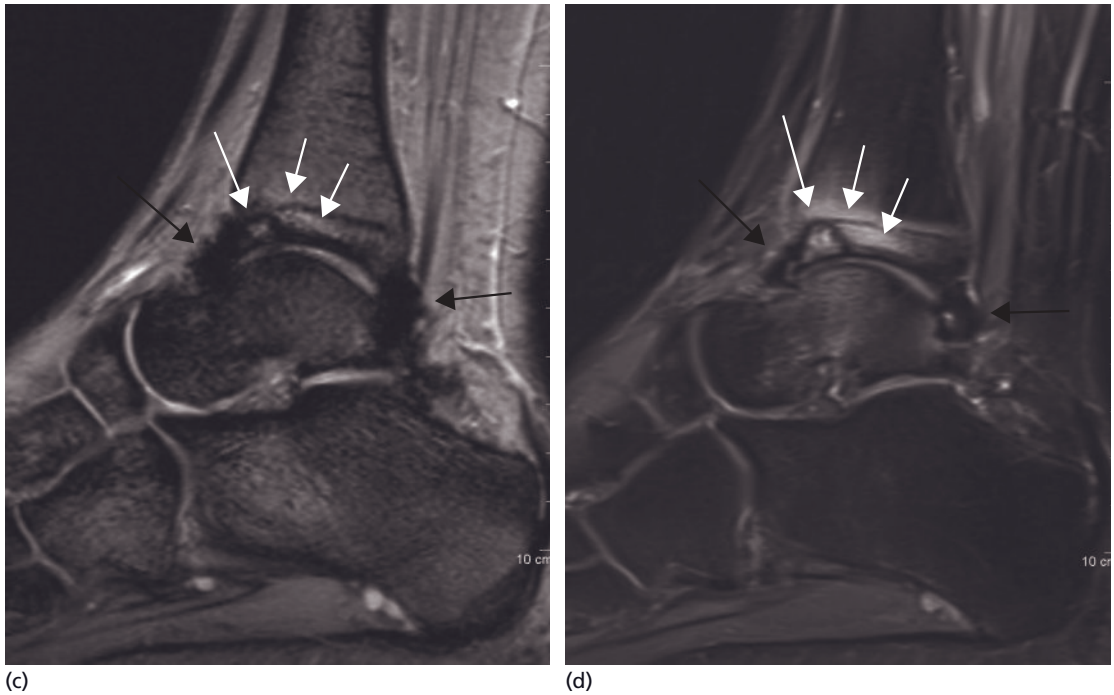
MRI visualizes early arthropathic changes such as hemarthroses, effusions, synovial hypertrophy, hemosiderin deposits, and small focal cartilage defects without joint space narrowing, none of which are easily discerned by X-ray imaging. Furthermore, compared with X-ray investigation, MRI can, in many cases, provide more detailed information about more advanced changes, such as erosions, subchondral cysts, and cartilage destruction with joint space narrowing (Figure 30.2). MRI is not only more effective than X-ray imaging for detecting changes in joints after clinical bleeds, but it has also been shown to more often reveal changes in joints with no clinical evidence of hemarthroses, which indicates that subclinical bleeds are more common than previously assumed [20,21].

MRI is useful for both clinical and research purposes. In equivocal cases of joint swelling, MRI can differentiate intra- and periarticular bleeds, effusion, and synovial hypertrophy even if ultrasonography can be a more cost-effective alternative. MRI can be helpful if data on factor level, anamnesis of hemarthroses, and physical examination are disproportionate. Joint changes as a result of subclinical bleeds in a patient with low factor levels but no or few known bleeds can indicate undertreatment and motivate introduction or increase of prophylaxis, and diagnostic work-up of a disproportionately symptomatic joint may reveal internal derangement such as ligament or meniscal tears or loose bodies, or other changes not necessarily related to hemophilia. Before performing synovectomy in therapy-resistant target joints, MRI can confirm the presence of hypertrophic synovia and exclude alternative explanations for the numerous bleeds. It would be highly beneficial if MRI could be employed to investigate the effectiveness of modern prophylactic factor regimens. The potential of MRI as an outcome measure in clinical trials has been demonstrated in a North American study comparing

Figure 30.1 Discrete osteopenia and slight epiphyseal enlargement representing mild arthropathic changes seen in frontal (A) and lateral (B) X-ray views of the left knee in a 12-year-old boy with severe hemophilia B. More severe arthropathic changes including soft-tissue swelling of suprapatellar bursa, epiphyseal enlargement, an irregular subchondral surface, narrowed joint space, cysts, and joint deformity are seen in X-ray images (C and D) of the right knee of a 33-year-old man with severe hemophilia A.



(a) (b)



(c) (d)

on-demand with prophylactic factor treatment [21]. The main difficulty with MRI assessment of hemophilic arthropathy is the long scan time required to image multiple joints, which makes the procedure unpractical and strenuous for the patient. Several different MRI scales for hemophilic arthropathy have been described in the literature [14–19,22], and in 2005 the International Prophylaxis Study Group (IPSG) presented a preliminary comprehensive scoring scheme [16,23] that combined the pioneer Denver MRI scale [14] and the more detailed European MRI score [15]. Further IPSG work is currently in progress with the aim of presenting a single scale for MRI scoring of hemophilic arthropathy.

Ultrasonography

Ultrasonography is a technique that measures the reflection or transmission of ultrasonic waves. Computer calculation of the distance to the sound-reflecting or absorbing surface plus the known orientation of the sound beam gives a two-dimensional image which is currently used in clinical practice. One of the first real-time sonographic images was obtained by the surgeon John Wild at Middlesex Hospital in London in 1954 of a 7-mm cancer of the breast nipple [24]. In the following decades, several technical implementations on ultrasonography were accomplished including the development of high-frequency transducers and color and power Doppler techniques [25]. The higher the frequency of an ultrasound transducer, the higher the spatial resolution of the corresponding image that is achieved, at the price of loss of capability of imaging deeper structures [26]. Linear high-resolution probes are typically used (7–13 MHz) for assessing hemophilic joints [27], enabling the visualization of superficial musculoskeletal structures such as synovium, tendons, musculature, and the cartilage/osteochondral interface at the edge of the joints on grayscale sonograms. In addition, power Doppler sonography has the capability of measuring synovial vascularity in hemophilic joints. A recent study [28] has shown that measurements obtained with this technique correlate strongly with dynamic contrast-enhanced MRI measurements.

Given some limitations of the ultrasound technique, such as operator dependency, and partial visualization of the joints (superficial structures are extremely well seen in detriment of deeper structures which lie away from the imaging field of view), the potential for this technique to assess hemophilic arthropathy has not been fully evaluated, and therefore this technique has been underutilized in clinical practice. Ultrasound has advantages over MRI, including lower cost of the examination, easier access in the clinical environment, no need for sedation in young children, and lack of interference of susceptibility artifacts which are commonly seen on gradient-echo MRI sequences of hemophilic joints with hemosiderin deposition. Susceptibility MRI artifacts are represented by low signal intensity ("blooming") that covers areas of hemosiderin deposition within the joint [22]. These artifacts may obscure the synovium in the joint. Therefore, if a given patient had previous intra-articular bleeds and now presents with significant hemosiderin deposition, gradient-echo MRI sequences cannot tell whether the patient has mild, moderate, or large synovial hypertrophy. The ultrasound technique, on the other hand, does not generate this artifact and therefore is able to quantitate the amount of synovial hypertrophy in the joint regardless of the amount of the hemosiderin deposition in this joint (Figure 30.3). This advantage of ultrasound over MRI is particularly important for evaluating the synovium status in hemophiliacs who are candidates for radiosynovectomy.

A technical challenge for acquisition of ultrasound images is the positioning of the transducer (angle of the ultrasound beam) on the patient's skin [27]. Since the images are generated by the sonographer who performs the examination, an inadequate position of the transducer may generate false-positive results for cartilage thinning, e.g., if the angle between the transducer and the horizontal plane lying over the joint becomes too small (Figure 30.4). Currently, few systematic protocols for ultrasound imaging acquisition of hemophilic joints are available in the literature [27]. Without systematization of imaging acquisition protocols it will not be possible to compare results of multicentric clinical trials performed in different institutions across the world in the future. Another current challenge for this technique relates to the interpretation of images. At this point, we are unaware of the sensitivity loss of the ultrasound technique compared with MRI with regard to the extent of tissues that can be evaluated by both imaging modalities. As such, although ultrasound evaluates superficial cartilage and osteochondral interfaces with great accuracy, it is unable to penetrate the deep tissues to provide information about the integrity of the central aspect of the

Figure 30.2 (A) Sagittal turbo-spin echo T2-weighted MR image (same knee as in Fig. 30.1A and B) revealing effusion (white arrows) and a small amount of synovial hypertrophy with hemosiderin deposits (black arrow). (B) A turbospin echo T2-weighted sagittal image (same knee as in Fig. 30.1C and D) showing large amounts of hemosiderin-laden hypertrophic synovia (large white arrows), cysts (black arrows) with loss of overlying cartilage, and bone erosion (small white arrows). (C) Sagittal gradient echo T2*-weighted image and corresponding (D) turbospin echo fat-suppressed T2-weighted image of left ankle in 16-year-old boy with severe hemophilia A showing synovial hypertrophy with hemosiderin deposits (black arrows), a subchondral cyst (large white arrow), and high signal bone edema (small white arrows). Note that hemosiderin is more conspicuous in the gradient echo T2*-weighted image because of artefactual "blooming," and, on the other hand, the subchondral cyst and the bone edema are best visualized in the turbospin echo fat-suppressed T2-weighted image.

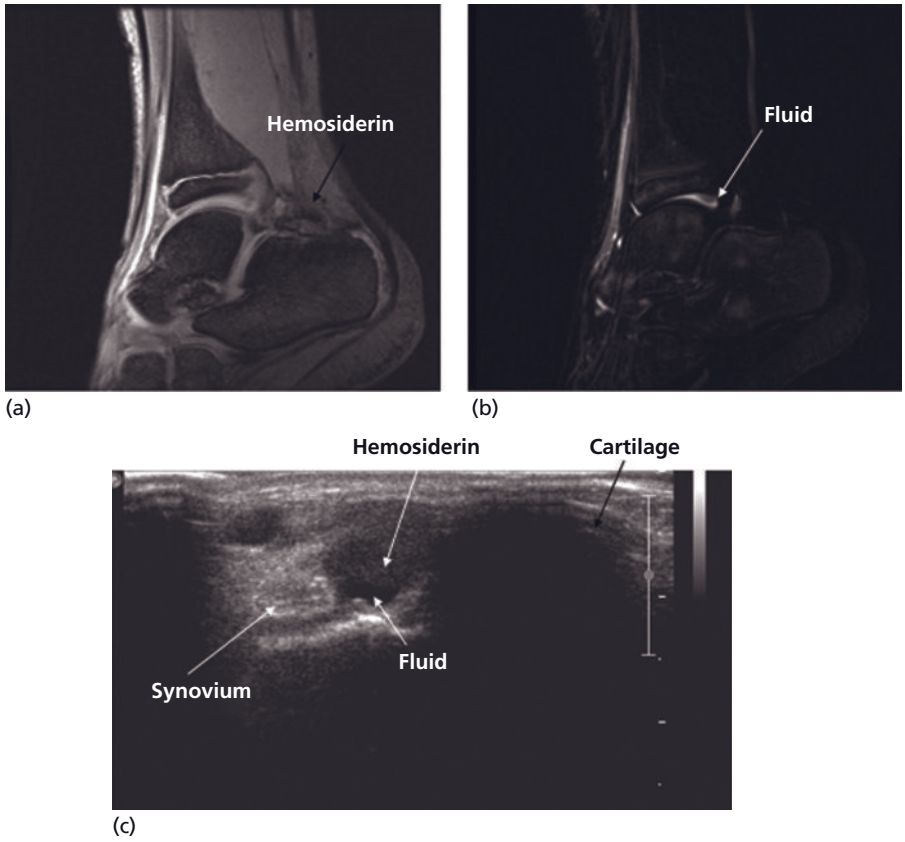


Figure 30.3 Sagittal gradient-echo (a) and T2-weighted (b) images of a hemophilic ankle show a mild amount of hemosiderin deposition and joint effusion. Only minimal "blooming" (susceptibility artifact) is noted within the posterior synovial recess of the ankle; however, the synovium is not promptly identified on the gradient-echo images. A corresponding sonogram demonstrates the conspicuity of the sonographic technique which allows easy discrimination between synovium (echogenic, "white"), hemosiderin (hypoechoic, "gray"), and fluid (anechoic, "black").

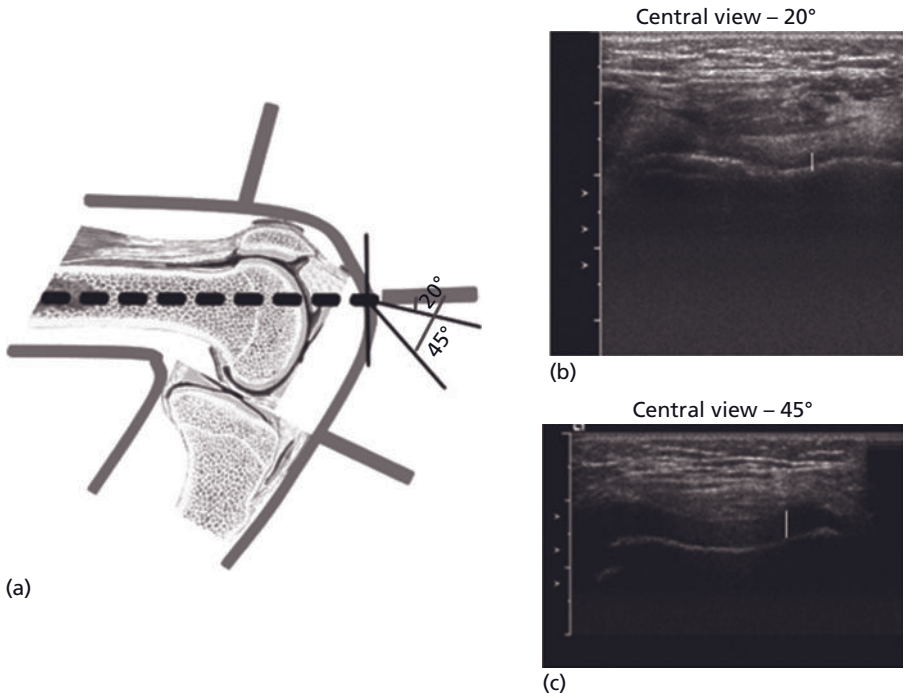


Figure 30.4 Axial ultrasound images used to assess cartilage thickness of the distal femur in a hemophilic patient. The transducer position is illustrated in (a) and the corresponding ultrasound images for the same knee are shown for 20° angulation (b) and 45° angulation of the ultrasound transducer (c). The cartilage thickness is marked on both sonographic images with a short line. Reproduced from [27] with permission.

articular cartilage or the posterior patellar cartilage in the knee joint, for example. Moreover, no prior studies are available on the normal imaging appearance of ossification centers in the ankle according to the expected child's joint growth. An incorrect interpretation of normal findings at different age groups of patients may lead to false-positive results for cartilage degeneration in unaffected hemophilic joints. Finally, great efforts have been made by different groups of investiga-

tors across the world to develop MRI scales for assessment of hemophilic arthropathy [14–19]. However, few ultrasound scales have been devised for evaluation of hemophilic joints [29]. The development of a universal ultrasound scale for evaluation of soft tissues (synovial hypertrophy, hemosiderin deposition, and joint effusion/hemarthrosis) and osteochondral tissues (changes in subchondral bone, joint margins, and cartilage loss) is ongoing. By comparing ultrasound and MRI on a head-by-head basis, it should be possible to quantitate the diagnostic sensitivity loss of ultrasound (for central aspects of the joint which affects especially the interpretation of cartilage loss and subchondral abnormalities). Further investigation will be required to determine whether the information missed by ultrasound is relevant for the clinical follow-up of hemophilic patients.

Novel imaging techniques

Most of the techniques described below are experimental or have been tested only in a small number of hemophilic patients; however, they hold promise for early assessment of hemophilic arthropathy in the future upon completion of their clinical validation.

Magnetic resonance imaging

Blood oxygen level-dependent (BOLD) magnetic resonance imaging

This is a noninvasive diagnostic technique that assesses the concentration of oxy- and deoxyhemoglobin at the capillary level during functional activation by comparing the response of the tissue to hyperoxia stimulus. An activated (hyperoxic) state increases the concentration of oxyhemoglobin, thus reducing the concentration of deoxyhemoglobin, which serves as an intrinsic, oxygen-sensitive, paramagnetic marker [30]. This technique detects temporal changes in the synovial response of the joint to a stimulus [31] and holds the potential to predict future synovial changes at an early stage of hemophilic arthropathy.

Ultrasmall superparamagnetic iron-oxide (USPIO) contrast-enhanced magnetic resonance imaging

Ultrasmall superparamagnetic iron-oxide (USPIO) particles have the property of superparamagnetism, which provides signal loss on gradient-echo images [32]. Previous studies [32] showed promising MRI results using USPIO particles to evaluate phagocytic macrophage activity in experimental animal models of arthritis. Because of their small size, USPIO particles are able to extravasate through capillary pores and are well suited for targeting synovial macrophages [33]. A technique capable of *in vivo* detection of phagocytic-active macrophages by means of iron oxide-associated signal effects may be a useful tool for surveillance of therapy. Preliminary studies on USPIO-DCE MRI conducted by our group of investigators demonstrated the feasibility of the technique in a rabbit model of hemophilia (unpublished data).

T1 mapping

Gadolinium-DTPA-enhanced T1 imaging is a technique sensitive to the cartilage proteoglycan content [34], and has been widely used on *in vivo* and *ex vivo* tissues. This technique utilizes the negative charge of the paramagnetic contrast agent which is assumed to distribute into cartilage inversely to the fixed charge density of glycosaminoglycans [34]. Thus, T1 relaxation time in presence of the contrast agent is approximately linearly related to the glycosaminoglycan content. This technique holds potential for detecting early cartilaginous changes (degeneration of glycosaminoglycans) prior to macroscopic visualization of cartilaginous damage in hemophilic joints.

T2 mapping

Quantitative T2 mapping of articular cartilage is a noninvasive imaging technique that has the potential to characterize hyaline articular cartilage and repair tissue in hemophilic joints. Alteration in the orderly transition in T2 values within cartilage has been shown to correlate to changes in water content and changes in collagen structure and organization associated with hyaline articular cartilage degradation [35,36]. This technique could serve as a proxy of collagen organization in the articular cartilage in hemophilic joints.

Ultrasonography

High-resolution/ultrasound biomicroscopy (UBM)

The development of higher frequency probes (13–20 MHz) has allowed the evaluation of skin thickness [37], hyaline cartilage, and intra-articular fibrocartilages and ligaments [38], and the assessment of cartilaginous changes undetectable macroscopically in arthritic joints [39]. Intra-articular high-resolution probes may also be useful in the intraoperative assessment of hemophilic joints (synovectomy), demonstrating the real-time microscopic status of the articular cartilage during the surgical procedure.

Contrast-enhanced ultrasonography

Intravenous microbubble echo-contrast agents have the potential to further increase the sensitivity of the color and power Doppler signal by raising the intensity of weak signals to a detectable level and are specially suitable for measuring and detecting changes of low-volume, low-velocity blood flow in small vessels such as those of the synovium in joints [40].

Positron emission tomography (PET)

^{18}F-FDG is an analogue of glucose labeled with a positron-emitting isotope, fluorine-18 (^{18}F), which is the main radiopharmaceutical used for PET. The degree of cellular FDG uptake is related to the cellular metabolic rate and the number of glucose transporters [41]. Imaging inflammation with ^{18}F-FDG-PET is based on the fact that infiltrated granulocytes and tissue macrophages use glucose as an energy source. When it is activated (inflammation), metabolism and thus FDG uptake increases [42]. Since hemophilia results in a noninflammatory or minimally inflammatory arthritis, the use of FDG PET/CT imaging techniques to characterize early physiologic processes in hemophilic arthropathy merits further research investigation.

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References

1 Resnick D. *Diagnosis of Bone and Joint Disorders*, 4th edn. Philadelphia: Saunders, 2002.
2 Arnold WD, Hilgartner MW. Hemophilic arthropathy. Current concepts of pathogenesis and management. *J Bone Joint Surg Am* 1977; **59**: 287–305.
3 Pettersson H, Gilbert M. *Diagnostic Imaging in Hemophilia*. Berlin: Springer-Verlag, 1985.
4 De Palma AF, Cotler JM. Hemophilic arthropathy. *AMA Arch Surg* 1956; **72**: 247–50.
5 Wood K, Omer A, Shaw MT. Haemophilic arthropathy. A combined radiological and clinical study. *Br J Radiol* 1969; **42**: 498–505.
6 Pettersson H, Ahlberg A, Nilsson IM. A radiologic classification of hemophilic arthropathy. *Clin Orthop Relat Res* 1980; **149**: 153–9.
7 Aledort LM, Haschmeyer RH, Pettersson H. A longitudinal study of orthopaedic outcomes for severe factor-VIII-deficient haemophiliacs. The Orthopaedic Outcome Study Group. *J Intern Med* 1994; **236**: 391–9.
8 Nilsson IM, Berntorp E, Lofqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. *J Intern Med* 1992; **232**: 25–32.
9 Manco-Johnson MJ, Pettersson H, Petrini P, *et al*. Physical therapy and imaging outcome measures in a haemophilia population treated with factor prophylaxis: Current status and future directions. *Haemophilia* 2004; **10**(Suppl. 4): 88–93.
10 Edelman RR, Hesselink JR, Zlatkin MB, Crues JV, III. *Clinical Magnetic Resonance Imaging*, 3rd edn. Philadelphia: Saunders, 2006.
11 Kulkarni MV, Drolshagen LF, Kaye JJ, *et al*. MR imaging of hemophiliac arthropathy. *J Comput Assist Tomogr* 1986; **10**: 445–9.
12 Rand T, Trattnig S, Male C, *et al*. Magnetic resonance imaging in hemophilic children: Value of gradient echo and contrast-enhanced imaging. *Magn Reson Imaging* 1999; **17**: 199–205.
13 Yulish BS, Lieberman JM, Strandjord SE, Bryan PJ, Mulopulos GP, Modic MT. Hemophilic arthropathy: Assessment with MR imaging. *Radiology* 1987; **164**: 759–62.
14 Nuss R, Kilcoyne RF, Geraghty S, *et al*. MRI findings in haemophilic joints treated with radiosynoviorthesis with development of an MRI scale of joint damage. *Haemophilia* 2000; **6**: 162–9.
15 Lundin B, Pettersson H, Ljung R. A new magnetic resonance imaging scoring method for assessment of haemophilic arthropathy. *Haemophilia* 2004; **10**: 383–9.
16 Lundin B, Babyn P, Doria AS, *et al*. Compatible scales for progressive and additive MRI assessments of haemophilic arthropathy. *Haemophilia* 2005; **11**: 109–15.
17 Funk MB, Schmidt H, Becker S, Escuriola C, Klarmann D, Klingebiel T, Kreuz W. Modified magnetic resonance imaging score compared with orthopaedic and radiological scores for the evaluation of haemophilic arthropathy. *Haemophilia* 2002; **8**: 98–103.
18 Soler R, López-Fernández F, Rodríguez E, Marini M. Hemophilic arthropathy. A scoring system for magnetic resonance imaging. *Eur Radiol* 2002; **12**: 836–43.
19 Dobón M, Lucía JF, Aguilar C, *et al*. Value of magnetic resonance imaging for the diagnosis and follow-up of haemophilic arthropathy. *Haemophilia* 2003; **9**: 76–85.
20 Lundin B, Ljung R, Pettersson H. MRI scores of ankle joints in children with haemophilia—comparison with clinical data. *Haemophilia* 2005; **11**: 116–22.
21 Manco-Johnson MJ, Abshire TC, Shapiro AD, *et al*. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; **357**: 535–44.
22 Kilcoyne RF, Lundin B, Pettersson H. Evolution of the imaging tests in hemophilia with emphasis on radiography and magnetic resonance imaging. *Acta Radiol* 2006; **47**: 287–96.
23 Doria AS, Babyn PS, Lundin B, *et al*. Reliability and construct validity of the compatible MRI scoring system for evaluation of haemophilic knees and ankles of haemophilic children. Expert MRI working group of the international prophylaxis study group. *Haemophilia* 2006; **12**: 503–13.
24 Shampo MA, Kyle RA. Stamp vignette on medical science. John Julian Wild—pioneer in ultrasonography. *Mayo Clin Proc* 1997; **72**: 234.
25 Rubin JM, Bude RO, Carson PL, Bree RL, Adler RS. Power Doppler US: A potentially useful alternative to mean frequency-based color Doppler US. *Radiology* 1994; **190**: 853–6.
26 Pavlin C. Ultrasound biomicroscopy high-frequency ultrasound imaging of the eye at microscopic resolution. *Radiol Clin North Am* 1998; **36**: 1047–58.
27 Zukotynski K, Jarrin J, Babyn PS, Carcao M, Pazmino-Canizares J, Stain AM, Doria AS. Sonography for assessment of haemophilic arthropathy in children: A systematic protocol. *Haemophilia* 2007; **13**: 293–304.
28 Acharya SS, Schloss R, Dyke JP, Mintz DN, Christos P, Dimichele DM, Adler RS. Power doppler sonography in the diagnosis of hemophilic synovitis—a promising tool. *J Thromb Haemost* 2008; **6**: 2055–61.

29 Klukowska A, Czyrny Z, Laguna P, Brzewski M, Serafin-Krol MA, Rokicka-Milewska R. Correlation between clinical, radiological and ultrasonographical image of knee joints in children with haemophilia. *Haemophilia* 2001; **7**: 286–92.
30 Lythgoe DJ, Williams SC, Cullinane M, Markus HS. Mapping of cerebrovascular reactivity using BOLD magnetic resonance imaging. *Magn Reson Imaging* 1999; **17**: 495–502.
31 Doria AS, Dick P. Region-of-interest-based analysis of clustered BOLD MRI data in experimental arthritis. *Acad Radiol* 2005; **12**: 841–52.
32 Dardzinski BJ, Schmithorst VJ, Holland SK, *et al*. MR imaging of murine arthritis using ultrasmall superparamagnetic iron oxide particles. *Magn Reson Imaging* 2001; **19**: 1209–16.
33 Mosher TJ. Imaging of rheumatoid arthritis: Can MR imaging be used to monitor cellular response of disease? *Radiology* 2004; **233**: 1–2.
34 Nieminen MT, Rieppo J, Silvennoinen J, Toyras J, Hakumaki JM, Hyttinen MM, Helminen HJ, Jurvelin JS. Spatial assessment of articular cartilage proteoglycans with Gd-DTPA-enhanced T1 mapping. *Magn Reson Med* 2002; **48**: 640–8.
35 Dardzinski BJ, Mosher TJ, Li S, Van Slyke MA, Smith MB. Spatial variation of T2 in human articular cartilage. *Radiology* 1997; **205**: 546–50.
36 Glaser C. New techniques for cartilage imaging: T2 relaxation time and diffusion-weighted MR imaging. *Radiol Clin North Am* 2005; **43**: 641–53.
37 Cosnes A, Anglade MC, Revuz J, Radier C. Thirteen-megahertz ultrasound probe: Its role in diagnosing localized scleroderma. *Br J Dermatol* 2003; **148**: 724–9.
38 Patonay L, Nagy K, Engelke W. Real-time endoarticular ultrasound imaging of the TMJ—a new diagnostic possibility? A cadaver study. *Int J Oral Maxillofac Surg* 2002; **31**: 553–7.
39 Doria AS, Babyn PS, Mendes M, Jong R, Pritzker K, Foster FS. Articular cartilage abnormalities of the knee in an antigen-induced arthritis model: Diagnostic efficacy of 40 MHz ultrasound biomicroscopy (UBM) (abstract). *Pediatr Radiol* 2003; **33**(Suppl. 1): S90.
40 Doria AS, Kiss MH, Lotito AP, Molnar LJ, de Castro CC, Medeiros CC, Cerri GG. Juvenile rheumatoid arthritis of the knee: Evaluation with contrast-enhanced color Doppler ultrasound. *Pediatr Radiol* 2001; **31**: 524–31.
41 Pauwels EK, Ribeiro MJ, Stoot JH, *et al*. FDG accumulation and tumor biology. *Nucl Med Biol* 1998; **25**: 317–22.
42 Love C, Tomas MB, Tronco GG, Palestro CJ. FDG PET of infection and inflammation. *Radiographics* 2005; **25**: 1357–68.

31 Physiotherapy in the management of hemophilia

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Introduction

The successful management of patients with hemophilia requires a coordinated multidisciplinary team approach that involves many professionals including doctors, nurses, counselors, scientists, and physiotherapists. Good communication between team members is essential to provide optimal patient outcome and a thorough understanding of each other's role is crucial. In the UK, the physiotherapist is an autonomous practitioner responsible for the physical health and well-being of patients and can offer assessment, treatment based on physical methods, advice, and education. Ideally, one physiotherapist should have sole responsibility for providing the physiotherapy service so enabling mutual trust and understanding to develop between the hemophilic patient and physiotherapist. The physiotherapist must have a thorough understanding of hemophilia including the medical management as well as the pathophysiologic effects of bleeding episodes and the development of arthropathy. Owing to the complex presentations of many patients, the physiotherapist must possess advanced problem-solving skills and be equipped with a broad range of assessment and management techniques, drawing on current evidence where available. The physiotherapy service should be located in the hemophilia center or with easy access to the rest of the hemophilia team. Close links with local physiotherapy services also need to be developed for those patients who live too far to attend the hemophilia center for physiotherapy on a regular basis.

This chapter will begin by discussing the physiotherapy assessment and management approaches for adults and children with hemophilia. Key issues relevant to the physiotherapy management of patients with inhibitors and acquired hemophilia will then be considered. Finally, the outcome measures available to evaluate physiotherapy interventions so that robust evidence of effectiveness can be provided will be highlighted.

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Adults with hemophilia

All patients who present with musculoskeletal problems should be referred to the physiotherapist. Depending on local arrangements, patients may also be able to self-refer directly to the physiotherapist, and an assessment is made [1]. The physiotherapist will undertake the initial assessment within a clinical reasoning framework drawing on evidence as appropriate. This assessment will establish baseline data regarding the patient's problem; determine the source of the problem, any contributory factors, the presence of precautions or contraindications to physiotherapy; and identify the patient's goals and expectations for physiotherapy management. The emphasis on a shared partnership between the patient and physiotherapist in determining the patient's management is increasingly being recognized [2].

Physiotherapy strategies

The adult with hemophilia may present with an acute joint or muscle bleed, chronic synovitis, or arthropathy. The typical clinical presentation of acute bleeds, chronic synovitis, and arthropathy, aims of treatment, and possible physiotherapy strategies for each condition is outlined in Table 31.1.

The effectiveness of factor replacement in the treatment of bleeds and the increasing use of home treatment programs means that many patients do not present to a hemophilia center when they experience a bleeding episode. However, this emphasis on self-management with a reliance on factor replacement only may mean that other strategies, such as graded exercise programs and education, are not employed. This may lead to minor persistent dysfunctions that are not detected on standard clinical tests that may predispose the patient to further trauma and, ultimately, deterioration in physical status [3,4]. Restoration of range of movement, strengthening exercises, and proprioceptive training are being increasingly recognized as important areas to address in reducing bleeds and increasing function [5–7].

Patients may also present with musculoskeletal problems unrelated to hemophilia and it is important to keep an open

Table 31.1 Physiotherapy management of musculoskeletal problems in hemophilia.

	Clinical presentation	Aims of physiotherapy	Possible physiotherapy strategies
Acute joint bleeds	"Aura" Pain Swelling Limitation of movement Muscle inhibition	Relieve pain Reduce swelling Restore function to prebleed status Prevent recurrence of bleeds	Advice on appropriate rest Ice Elevation Electrotherapy Graded exercise program Modification of activities Advice and education
Acute muscle bleeds	Pain on palpation and stretching muscle Limitation of movement Bruising may be evident Muscle inhibition/spasm Neural compression	Relieve pain Reduce swelling Restore function to prebleed status Prevent recurrence of bleeds	Rest Ice Elevation Electrotherapy Graded exercise program with appropriate muscle stretching Modification of activities Advice and education
Chronic synovitis	Chronic effusion and thickening of the synovial membrane Minimal pain Severe muscle atrophy Poor proprioception	Reduce swelling Protect from further bleeding Increase muscle strength and endurance Improve coordination and proprioception Maximize function	Electrotherapy Supports/braces to protect joint in presence of muscle weakness Graded strengthening program and correction of muscle imbalances Proprioceptive retraining Hydrotherapy Advice and education
Arthropathy	Pain Loss of range of movement Muscle weakness Contractures Deformity Bony or fibrous ankylosis	Relieve pain Increase or maintain range of movement Increase or maintain muscle strength and endurance Improve coordination and proprioception Maximize function	Mobilization of joints Correction of muscle imbalances Hydrotherapy Splints and braces Electrotherapy including transcutaneous nerve stimulation for pain relief Advice and education

Based on Beeton [18].

mind and not assume that all dysfunction is a result of bleeding [8]. A patient may, for example, complain of neck pain because of poor seating at work. The thorough assessment process by the physiotherapist aims to identify whether or not the problem is related to hemophilia, and to manage this appropriately.

Classification of adult patients with hemophilia

Adults with hemophilia can be grouped into four main categories (see Table 31.2) and this can have implications for their possible clinical presentation and subsequent physiotherapy management.

Patients with mild and moderate hemophilia are less likely than severely affected patients to have physical problems because of their hemophilia, and will often partake in more vigorous physical activities and sports. However, these patients do not generally have experience of bleeding episodes and may ignore or not recognize initial bleeding problems. These patients may not be able to administer their own factor replacement and may not seek help until the bleeding has become severe. The patient should be encouraged to seek advice promptly if a traumatic incident occurs. Delays in administering factor replacement can lead to long-term problems; even pseudotumors have been reported [9]. All mildly and moderately affected patients should be reviewed at least on an annual basis by the physiotherapist to evaluate the musculoskeletal system and provide advice on appropriate physical activities.

Patients who have had long-term prophylaxis since a young age may have minimal joint dysfunction owing to the long-term benefits of factor replacement [10]. Interestingly, like the mildly affected patients, these patients may also have limited experience of bleeds and this may promote more risk-taking behavior. Some patients can present with a target joint and may experience symptoms as a result of acute bleeds or early arthropathy. The ankle joint, rather than the knee, is increasingly being identified as the joint most commonly affected, especially in younger patients, although the reasons for this remain uncertain [11]. Physiotherapy modalities, including

Table 31.2 Impact of use of factor replacement on physical status

Group	Use of factor replacement	Physical status
Group 1	Patients with mild/moderate hemophilia rarely have factor replacement	Few physical problems but can present late as may underestimate the effect of trauma
Group 2	Patients have been on long-term prophylaxis	Normal mobility Minimal joint dysfunction or dysfunction not detectable on standard physical tests
Group 3	Patients on secondary prophylaxis with limited treatment in childhood and early years	Marked joint dysfunction involving one or two joints May require joint replacement
Group 4	Patients may or may not have had prophylaxis, on-demand treatment, few—if any—bleeds now	Multiple joint involvement Often not much pain, but stiffness and marked disability May have had joint replacements in past

manual techniques and correction of lower limb biomechanics by use of custom-made inserts, have been recommended in reducing symptoms [12]. These patients may have few physical limitations but may have to restrict some activities to avoid symptoms or to prevent problems occurring. However, while the physical limitations may be minimal, the psychological impact of "appearing normal" but being unable to be involved in activities with peers may be considerable [13,14].

Patients who did not have access to factor replacement when they were younger may have marked musculoskeletal dysfunction in one or two joints which may be severely affected because of synovitis or arthropathy. The physiotherapist has a range of treatment strategies to offer depending on the patient's preferences, previous benefit from treatment, and available research evidence (see Table 31.1). Factor replacement is usually administered prior to physiotherapy. Although physiotherapy can be provided without factor cover with care, it does enhance confidence for both the patient and physiotherapist, particularly when introducing new techniques and exercise regimes. If, following physiotherapy treatment, the symptoms are unchanged, then the patient should be referred for an orthopedic opinion. Joint clinics attended by all members of the team can be very valuable as shared discussions with the patient can ensure the most appropriate management strategy.

The final group of patients, who are often older, may have developed multiple joint arthropathy and demonstrate marked disability. Some of these patients may have severe pain and others little, if any, pain, although all patients are likely to have limited range of movement, deformity, and contractures. Their joints may bleed only a little, if at all. The aim of treatment is focused on maximizing function and adapting to limitations with appropriate strategies such as pacing activities, exercise programs, and avoiding aggravating activities. If the limitation of movement is as a result of contractures and the end feel of the joint is hard and bony, manual physiotherapy techniques may have limited benefit. Patients often learn appropriate strategies to manage their limitations and in doing so mask their disability. It is important to discuss the strategies that patients use. The physiotherapist may be able to suggest other strategies and can also advise on exercise programs and appropriate activities to maintain physical fitness and cardiovascular function. In addition, patients may be at risk of osteoporosis because of inactivity as a result of hemophilic arthropathy [15]. For patients with severe pain not helped by physiotherapy, orthopedic surgery can offer considerable improvements in pain and function and improve quality of life [16]. The physiotherapist plays an important role in the pre- and postoperative periods in order to assist the patient in achieving optimal recovery [17].

Children with hemophilia

The main aim of the physiotherapy management of children with hemophilia is to minimize the long-term musculoskeletal effects of hemophilia. This can be achieved by:

- early detection and physiotherapy treatment of bleeds;
- offering education and support to enable children and their parents to have a thorough understanding of the condition; and
- advising on appropriate activity and sport to reduce the risk of bleeds.

Advances in early identification of joint damage in children have provided physiotherapists with the opportunity of early intervention. Particular advances include the Haemophilia Joint Health Score [18], developed to detect more minor joint changes that tend to occur in younger patients, and a single international MRI scoring system to detect and assess mild structural joint changes [19]. Early changes in gait patterns in boys with severe hemophilia have also been identified using an electronic pressure-sensitive walkway. This device has appeared sensitive enough to identify subclinical changes in the gait pattern occurring in asymptomatic children as well as those with arthropathy [3].

Primary prophylaxis has been recommended by the World Federation of Hemophilia (WFH) as the optimum treatment for children [20] as the effects of bleeding in young joints can be considerable [21]. However, prophylaxis can be demanding, particularly for very young patients [22], and more recently an individualized and tailored regime according to the bleeding pattern has been recommended [23].

The physical assessment will identify the musculoskeletal dysfunctions that need to be addressed in order to devise an

appropriate individualized exercise regime based on the goals and expectations of the child and their parents. Where the expectations and goals are inappropriate then a careful explanation should be offered and if necessary the involvement of an experienced counselor may be required [24].

Hydrotherapy has been shown to have positive effects in reducing bleeding frequency, pain, and instability in a target joint and also improving range of movement and muscle girth [25]. It is a stimulating environment for most children and an excellent aerobic exercise; however, it may entail some risk for patients with severe hemophilia who experience hemarthroses in their elbows and shoulders [26].

The use of electrotherapy modalities, particularly ultrasound and pulsed shortwave, in children is an area where there is controversy. It is a widely held view that it is acceptable to treat children with electrotherapy. However, there is a theoretical risk that some modalities of electrotherapy may affect the epiphyseal growth plates and that it should be avoided where there is an active bleed or the possibility of a re-bleed. It is important to determine whether the theoretical risk of electrotherapy outweighs the risks of not using electrotherapy and the joint dysfunction that may ensue [27].

In a study of Dutch children with moderate or severe hemophilia on prophylaxis, it was identified that they were comparable with healthy children in terms of joint motion, muscle strength, motor performance, and skills in activities of daily living [13]. Therefore, physical exercise and sports should be encouraged. The benefits and risks of different types of exercise need to be considered, as more vigorous activity may carry risk of trauma, but this must be balanced against benefits to the musculoskeletal system [28].

The National Haemophilia Foundation and the WFH have produced guidelines for physical activity programs, and the safety of sports has been classified according to risk [29]. An orthopedic examination and five-item fitness check has been developed to assess physical ability so that the most appropriate sport can be chosen for the individual. Care needs to be taken to avoid introducing children to activities that place undue stress and strains upon their joints whilst recognizing that activity choices can be expanded as children age and coordination and strength develop [30].

In developing countries where factor replacement and access to specialized assessment tools are not available, it is still important to exercise as there is some evidence that exercise increases levels of circulating blood factor [31]. Exercise also has other benefits and although the choice of sports that children can participate in may be limited, activities should still be encouraged. Some hemophilia societies operate summer camps aiming to build children's self-confidence, encourage independence, build friendships, develop leadership skills, and instill team-building qualities [32].

The World Health Organization has declared obesity a global epidemic. Sedentary lifestyles are becoming more widespread; individuals are spending greater amounts of time watching television, using computers, and using "passive" modes of transportation [33]. Obesity in boys with hemophilia has tripled in the last few years. This can aggravate symptoms of arthropathy and may predispose to cardiac disease and affect quality of life. This needs to be addressed and measures put in place to prevent weight gain [34].

Patients with inhibitors/ acquired hemophilia

This section will discuss some of the key issues that need to be considered when undertaking physiotherapy for patients with inhibitors and acquired hemophilia. Patients with inhibitors present a particular challenge to all members of the multidisciplinary team [35]. It is, therefore, particularly important that the physiotherapist has considerable knowledge of the manifestations of hemophilia and possible medical treatment options before managing these patients. It has been reported that these patients may experience more pain and disability than other patients with hemophilia because of poor control of bleeding [36] and therefore assessment by a physiotherapist is even more crucial. However, concerns about the ease with which spontaneous bleeds may occur may deter members of the team from referring to the physiotherapist and physical dysfunction and pain that could be improved may go unmanaged.

The physiotherapist may be the first person to query whether a patient may have developed an inhibitor. Careful questioning will alert the physiotherapist that the bleed is not resolving or the patient may be complaining of frequent bleeds in spite of regular and adequate factor replacement, and a medical opinion should be sought [1]. Once the patient has been diagnosed with an inhibitor there is much that the physiotherapist can offer. Bleeds may or may not be treated with factor replacement depending on whether the patient has a high- or low-titer inhibitor (measured in Bethesda units) and whether the patient is a low or high responder [37]. All patients must be managed with considerable care as the type of inhibitor present can change over time [37] and it is always essential to avoid causing bleeds or exacerbating further bleeding episodes.

Following an acute muscle or joint bleed, a very limited physical examination should be performed. This may include only observation of posture and extent of bruising, if any, and measurement of swelling and active pain-free movement. For the treatment of acute bleeds, synovitis, and arthropathy, similar techniques and modalities as used for adults and children with hemophilia can be employed provided that there is no active bleeding, all movements are pain free, and exercises are progressed cautiously. The key issue in the physiotherapy management for the patient with inhibitors is to avoid exacerbating the bleeding, therefore:

- it is particularly important to ensure the bleeding has stopped before starting physiotherapy;

• physiotherapy assessment and treatment should be carefully timed to occur when factor levels are highest if factor replacement is being administered;
• electrotherapy modalities should be used with care only when bleeding has ceased [27];
• a carefully controlled, graded exercise program should be followed, starting with isometric exercises with low repetitions and progressing slowly based on response to exercises and patient comfort; and
• high-resistance and high-impact exercises are contraindicated [35].

Many patients with long-term inhibitor problems will have multiple joint arthropathy as a result of uncontrolled bleeding. Surgery may not be a possibility for some of these patients and conservative measures may be the only option in relieving symptoms and maximizing function [38]. Advice and education are essential to help the patient develop strategies to minimize stress on joints and control pain. Simple exercise regimes to build up muscle strength and maintain, and if possible increase, mobility should be provided. Hydrotherapy can be a particularly useful adjunct as the buoyancy of the water can relieve weight bearing and enable the patient to move more freely. It is essential that patients do not overexercise in the hydrotherapy pool, particularly in the early stages of rehabilitation as the warm water and weightlessness can promote overconfidence. The patient needs to have an appropriate lifestyle and physical activities need to be chosen with care. Sports such as tai chi may still be enjoyed, facilitating muscle strength and good postural control as well as promoting the psychological benefits of exercise [39]. Some sports can be performed in sitting rather than standing positions, thus reducing the weight-bearing stresses on lower limb joints affected by arthropathy [40].

Patients with acquired inhibitors may develop severe bleeds often affecting the muscles. Left untreated, these muscle hematomas can lead to contractures and compartment syndromes [35]. The acquired inhibitor may have occurred secondary to other medical conditions such as rheumatoid arthritis. These additional medical problems need to be considered when planning treatment. Patients may be very frightened and anxious about the bleeding problem and may require a great deal of reassurance and appropriate explanations. Treatment is focused on managing the symptoms of bleeds using the strategies described above and providing advice on avoiding or minimizing problems in the future.

Outcome measures

It is important that an appropriate tool is chosen to evaluate the impact of physiotherapy in order to demonstrate the effectiveness of therapeutic interventions. It is essential, therefore, that the measures used are reliable, valid, and sensitive to change [41,42]. Three main types of outcome measure are available. These are impairment measures, functional measures, and quality of life measures.

Impairment measures

Measures of impairment include the evaluation of a range of physical testing procedures including posture, range of movement, and muscle strength. The advantage of using an impairment measure to evaluate outcome is that these measures are relatively easy and quick to perform. The disadvantages of these measures can be the poor reliability of measurements especially between different raters [42]. Changes in measures may also not correspond to changes in a patient's function or quality of life. The WFH scale has traditionally been used to measure impairment in hemophilia [43]; however, it does not appear to be sensitive enough to detect the more minor changes that tend to occur in younger patients. The Haemophilia Joint Health Score (HJHS) has been developed to address these concerns, and in a small inter-rater study it has demonstrated good reliability [18].

It is recommended that all patients with hemophilia are monitored over time. Ideally, a 6-month or, at the minimum, yearly review should be undertaken [44]. Physical impairment measures can be useful when evaluating the progression of hemophilic arthropathy. If deterioration in range of movement, strength, or other impairment measure is noted, physiotherapy interventions can be instigated to improve this.

Functional measures

These measures can be used to provide information on a patient's functional health status. The advantage of functional measures is that they assess the ability to undertake tasks of daily living that is usually of relevance to the patient as well as the physiotherapist. Two hemophilic specific functional measures are available. These are the Haemophilia Activities List (HAL), a self-assessment questionnaire which has undergone extensive psychometric testing [45], and the Functional Independence Score for Haemophilia (FISH), a performance measure [46]. Both of these instruments can be administered to patients as they measure different constructs of function [47].

Quality of life measures

A proliferation of hemophilia-specific quality of life questionnaires have been developed over recent years for both adults and children. Increasingly, patients have been involved in the development of these questionnaires, ensuring that the questions asked are relevant to patients [48]. Overall, studies have generally demonstrated lower quality of life scores in patients with hemophilia compared with normal population figures. However, a number of authors have suggested that scores were expected to be even lower than those achieved [49–51]. Various reasons have been proposed to explain this finding including adapting to condition, re-evaluating priorities, and coping strategies. This suggests caution of the use of standard-

ized quality of life questionnaires in isolation. Understanding the patient experience using interviews and focus groups may provide valuable information on the impact of hemophilia for an individual [52] and also for the families [14].

Conclusion

The physiotherapist needs to adopt a problem-solving, clinical-reasoning approach and to reflect on the outcomes when managing musculoskeletal dysfunction in patients with hemophilia. Patients with hemophilia are often experts and there is much to be learnt from listening and talking to the patient and developing appropriate treatment strategies based on mutually agreed goals. However, not all patients will necessarily have experience of hemophilia, such as patients with mild hemophilia or those who have recently developed an inhibitor or acquired hemophilia. Other patients may have denied their hemophilia and continued to adopt an inappropriate lifestyle. These patients need careful explanations, encouragement, and regular monitoring of their problems. Finally, a valid, reliable, and sensitive outcome measure must be used in order to provide an objective measure of the effectiveness of the physiotherapy treatment. Physiotherapists should be encouraged to disseminate their outcomes so enhancing the evidence base and promotion of best practice in the physiotherapy management of hemophilia.

References

1 Beeton K, Ryder D. Principles of assessment in haemophilia. In: Buzzard B, Beeton K (eds.) *Physiotherapy Management of Haemophilia*. Oxford: Blackwell Science, 2000.
2 Higgs J, Jones M. *Clinical Reasoning in the Health Professions*. Oxford: Butterworth Heinemann, 2000.
3 Bladen M, Alderson L, Khair K, Liesner R, Green J, Main E. Can early subclinical gait changes in children with haemophilia be identified using the GAITRite walkway. *Haemophilia* 2007; **13**: 542–7.
4 Gallach J, Querol F, Gonzalez L, Pardo A, Aznar J. Posturographic analysis of balance control in patients with haemophilic arthropathy. *Haemophilia* 2008; **14**: 329–35.
5 Hilberg T, Herbseb M, Puta C, Gabriel H, Schramm W. Physical training increases isometric muscular strength and proprioceptive performance in haemophilic subjects. *Haemophilia* 2003; **9**: 86–93.
6 Titinsky R, Falk B, Heim M, *et al*. The effect of resistance training on the frequency of bleeding in haemophilia patients: A pilot study. *Haemophilia* 2003; **8**: 22–7.
7 Harris S, Boggio L. Exercise may decrease further destruction in the adult haemophilic joint. *Haemophilia* 2006; **12**: 237–40.
8 Dalzell R. Non-haemotological pathologies—the importance of musculoskeletal assessment in haemophilia. *Haemophilia* 2005; **11**: 620–2.
9 Buzzard B. Trauma induced pseudotumours in two brothers with moderate factor IX deficiency. In: *Proceedings of the 4th Musculoskeletal Congress of the World Federation of Haemophilia*. Madrid, 1997.
10 Fischer K, Van der Bom J, Mauser-Bunschoten E, *et al*. Changes in treatment strategies for severe haemophilia over the last three decades: Effects on clotting factor consumption and arthropathy. *Haemophilia* 2001; **7**: 446–52.
11 Betsy M, Gilbert M. Haemophilic haemarthrosis. In: Rodriguez-Merchan EC (ed.) *The Haemophilic Joints: New Perspectives*. Oxford: Blackwell Publishing, 2003.
12 Stephensen D. Biomechanics of the lower limb in haemophilia. In: Buzzard B, Beeton K (eds.) *Physiotherapy Management of Haemophilia*. Oxford: Blackwell Science, 2000.
13 Schoenmakers M, Gulmans V, Helders P, Van Den Berg, H. Motor performance and disability in Dutch children with haemophilia: A comparison with their healthy peers. *Haemophilia* 2001; **7**: 293–8.
14 Beeton K, Neal D, Watson T, Lee CA. Parents of children with haemophilia—a transforming experience. *Haemophilia* 2007; **13**: 570–9.
15 Wallny T, Scholz D, Oldenburg J, *et al*. Osteoporosis in haemophilia—an underestimated comorbidity? *Haemophilia* 2007; **13**: 79–84.
16 Peacock K. Quality of life before and after surgery: Mobility issues, the fear of surgery, inpatient recovery and outpatient rehabilitation. *Haemophilia* 2005; **11**(Suppl. 1): 30–1.
17 de Kleijn P, Blamey G, Zourikian N, Dalzell R, Lobet S. Physiotherapy following elective orthopaedic procedures. *Haemophilia* 2006; **12**(Suppl. 3): 108–12.
18 Hilliard P, Funk S, Zourikian N, *et al*. Hemophilia joint health score reliability study. *Haemophilia* 2006, **12**: 518–25.
19 Doria AS, Babyn PS, Lundin B, *et al*. Reliability and construct validity of the compatible MRI scoring system for evaluation of haemophilic knees and ankles of haemophilic children. Expert MRI working group of the international prophylaxis study group. *Haemophilia* 2006; **12**: 503–13.
20 Ljung R. Aspects of haemophilia prophylaxis in Sweden. *Haemophilia* 2002; **8**: 34–7.
21 Jansen NWD, Roosendaal G, Bijlsma JWJ, DeGroot J, Theobald M, Lafeber FPJG. Degenerated and healthy cartilage are equally vulnerable to blood-induced damage. *Ann Rheum Disease* 2008; **67**: 1468–73.
22 Petrini P. Identifying and overcoming barriers to prophylaxis in the management of haemophilia. *Haemophilia* 2007; **13**(Suppl. 2): 16–22.
23 Biss TT, Chan AK, Blanchette VS, Iwenofu LN, Mclimont M, Carao MD. The use of prophylaxis in 2663 children and adults with haemophilia: Results of the 2006 Canadian national haemophilia prophylaxis survey. *Haemophilia* 2008; **14**: 923–30.
24 Miller R, Beeton K, Goldman E, Ribbans W. Counselling guidelines for managing musculoskeletal problems in haemophilia in the 1990s. *Haemophilia* 1997; **3**: 9–13.
25 Tiktinsky R, Barmatz C. Hydrotherapy and its use in haemophilia. In: Buzzard B, Beeton K (eds.) *Physiotherapy Management of Haemophilia*. Oxford: Blackwell Science, 2000.
26 McLain LG, Heldrich FT. Hemophilia and sports. Guidelines for participation. *Phys Sports Med* 1990; **76**: 77.
27 Watson T. Current concepts in electrotherapy. *Haemophilia* 2002; **8**: 413–18.
28 Froberg K, Lammert O. *Exercise and Fitness—Benefits and Risks in Children and Exercise*, VIII. Odense: Odense University Press, 1997.
29 Wittmeier K, Mulder K. Enhancing lifestyle for individuals with haemophilia through physical activity and exercise: the role of physiotherapy. *Haemophilia* 2007; **13**(Suppl. 2): 31–7.

30 Seuser A, Boehm P, Kurme A, Schumpe G, Kurnik K. Orthopaedic issues in sports for persons with haemophilia. *Haemophilia* 2007; **13**(Suppl. 2): 47–52.
31 Koch B, Luban NL, Galioto FMJ, Rick ME, Goldstein D, Kelleher JF, Jr. Changes in coagulation parameters with exercise in patients with classic haemophilia. *Am J Hematol* 1984; **16**: 227–33.
32 Buzzard B M. Physiotherapy, rehabilitation and sports in countries with limited replacement coagulation factor supply. *Haemophilia* 2007; **13**(Suppl. 2): 44–6.
33 Riske B. Sports and exercise in haemophilia: Benefits and challenges. *Haemophilia* 2007; **13**(Suppl. 2): 29–30.
34 Hofstede FG, Fijnvandraat K, Plug I, Kamphuisen PW, Rosendaal FR, Peters M. Obesity: A new disaster for haemophilic patients? A nationwide survey. *Haemophilia* 2008; **14**: 1035–8.
35 Beeton K, Buzzard B. Physiotherapy in the management of patients with inhibitors. In: Rodriguez-Merchan EC, Lee CA (eds.) *Inhibitors in Patients with Haemophilia*. Oxford: Blackwell Science, 2002.
36 Roberts H. Inhibitors and their management. In: *Haemophilia and Other Inherited Bleeding Disorders*. London: W.B. Saunders, 1997.
37 Dimichele D. Inhibitors in haemophilia: A primer. *Haemophilia* 2000; **6**(Suppl. 1): 38–40.
38 Rodriguez-Merchan EC, Quintana M, Jimenez-Yuste V. Orthopaedic surgery in patients with inhibitors as a last resort. *Haemophilia* 2008; **1**(Suppl. 6): 56–67.
39 Danusantoso H, Heijnen L. Tai Chi Chuan for persons with haemophilia. *Haemophilia* 2001; **7**: 437–8 (correspondence).
40 Heijnen L. The role of rehabilitation and sports in haemophilia patients with inhibitors. *Haemophilia* 2008; **14**(Suppl.6): 45–51.
41 Fitzpatrick R, Davey C, Buxton M, Jones, D. Evaluating patient-based outcome measures for use in clinical trials. *Health Technol Assess* 1998; **2**: 1–73.
42 Rodriguez-Merchan EC. Orthopaedic assessment in haemophilia. *Haemophilia* 2003; **9**: 65–74.
43 Pettersson H, Gilbert MS. Classification of the hemophilic arthropathy. In: Pettersson H, Gilbert MS (eds.) *Diagnostic Imaging in Hemophilia*. New York: Springer-Verlag, 1985: 56–68.
44 Haemophilia Alliance. *A National Service Specification for Haemophilia and Related Conditions*, 2001. http://www.haemophiliaalliance.org.uk/
45 van Genderen FR, Westers P, Heijnen L, *et al.* Measuring patients' perceptions on their functional abilities: Validation of the Haemophilia Activities List. *Haemophilia* 2006; **12**: 36–46.
46 Poonnoose PM, Thomas R, Keshava SN, *et al.* Psychometric analysis of the Functional Independence Score in Haemophilia (FISH). *Haemophilia* 2007; **13**: 620–6.
47 Beeton K, de Kleijn P, Hilliard P, *et al.* Recent developments in clinimetric instruments. *Haemophilia State of the Art* 2006; **12**(Suppl. 3): 102–7.
48 Young N, Bradley C, Blanchette V, *et al.* Development of a health-related quality of life measure for boys with haemophilia: The Canadian Haemophilia Outcomes—Kids Life Assessment Tool (CHO-KLAT). *Haemophilia* 2004; **10**: 34–43.
49 Rosendaal F, Smit C, Varekamp I, *et al.* Modern haemophilia treatment: Medical improvements and quality of life. *J Intern Med* 1990; **228**: 633–40.
50 Djulbegovic B, Goldsmith G, Vaughn D, *et al.* Comparison of the quality of life between HIV-positive haemophilia patients and HIV-negative haemophilia patients. *Haemophilia* 1996; **2**: 166–72.
51 Miners A, Sabin C, Tolley K, Jenkinson C, Kind P, Lee C. Assessing health-related quality-of-life in individuals with haemophilia. *Haemophilia* 1999; **5**: 378–85.
52 Beeton K, Neal D, Lee CA. An exploration of health related quality of life in adults with haemophilia—a qualitative perspective. *Haemophilia* 2005; **11**: 123–32.

32 Clinimetric instruments in hemophilia

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In the management of chronic incurable diseases, assessment of long-term outcome becomes particularly important, not only to document the impact of the condition on the individual, but also to compare different treatment strategies. In other chronic disorders that predominantly affect the joints, such as rheumatoid and osteoarthritis, various generic and disease-specific clinimetric instruments have been used for a long time—to assess not only joint structure, but also overall musculoskeletal function [1]. While the same approach should apply to patients with hemophilia, this has not been the case. Since the development of the clinical and the radiologic Pettersson score introduced by the Musculoskeletal Committee of the World Federation of Hemophilia (WFH) in the 1980s to assess joints affected by recurrent hemarthrosis, there has been very little progress [2,3]. In addition to being insensitive to early change, these scores fail to assess the impact of interventions on the overall health of the individual—in terms of activities of daily living, functional ability, schooling, work and social life, and quality of life [4]. Over the years, there has been a shift in the philosophy of outcome measurement, from the "biomedical model"—where changes in structure was the primary outcome measure, to an assessment in terms of the disablement process as conceptualized by the "biopsychosocial model" [5]. It has only been over the last 5 years that "disease-specific" tools to assess functional ability and quality of life have been developed to assess the other aspects of health that have previously not been addressed comprehensively [4]. With the array of tools available to assess the efficacy of therapeutic interventions, it is essential that the researcher is aware of the psychometric properties of the clinimetric instruments used, as well as their limitations.

Musculoskeletal assessment: outcome measurement

To assess the impact of disease on the health of the individual, two major conceptual frameworks have been used. The Disablement Process (DP), as described by Verbrugge and Jette, measures outcome in terms of four domains: *Pathology*, which refers to chemical and physiologic abnormalities detected; *Impairments*, which refers to dysfunction and structural abnormalities in specific body systems; *Functional Limitation*, which is restrictions in performing physical and mental actions used in activities of daily life; and *Disability*, which refers to the difficulty doing activities in any domain of life because of the health problem [5]. According to the International Classification of Functioning, Disability, and Health (ICF) one needs to assess the impact of the disease on *body structures and functions*, *activities*, and *participation* (Figure 32.1) [6]. Body structures are parts of the body such as organs, limbs, and their components. Body functions are defined as the physiologic functions of these systems, such as range of motion (ROM), strength, and instability. *Activities* involve executing tasks or actions, and a range of activities that can be performed by the individual are covered. *Participation* is defined as involvement in a life situation, such as sport, leisure, work, or social events. The ICF provides a single list of activities and participation in nine domains. According to their needs and purposes, investigators can designate some domains as activities and others as participation, without overlap; alternatively, they can designate all domains to be potentially both activities and participation, and use qualifiers to differentiate the data. The components of the list can be affected by *contextual* factors, which represent a person's background, and include both *environmental* and *personal* factors. Environmental factors comprise the physical, social, and attitudinal environment in which an individual lives and conducts day-to-day activities. They include social attitudes, architectural characteristics, and social and legal structures, as well as climate and terrain. Personal factors include aspects of an individual's life that are not necessarily part of the health condition or health status, such as age, sex, and indigenous status. The concept of *quality of life* (QoL) is complex, and embraces many characteristics of the social, economic, and physical environment, as well as the health and internal state of the individual [6].

Drawing parallels between the two frameworks, in order to measure outcome, one needs to assess the impact of the *Disease* (health condition/pathologic changes) on the individual's *Body* (in terms of impairments/changes in body function and structure), as well as in terms of the effect it has on

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the *Person*, in terms of his functional limitations and difficulty in performing activities of daily life. Equally important is the assessment of the impact of the disease on his involvement in *Society* (Figure 32.2). In keeping with this framework, the clinimetric instruments in hemophilia have been discussed under three broad sections: those that evaluate the structure and function of individual joints (the Body)—the clinical and radiologic score; those that assess the limitation in activities and functional independence—both subjectively and objectively (the Person); and those that assess his participation in life activities and quality of life (Society).

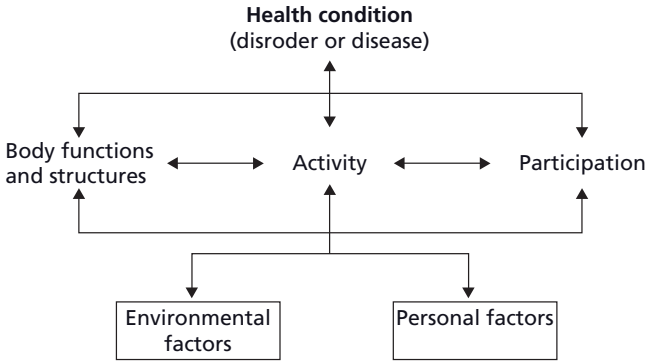

Figure 32.1 Interactions between the components of the International Classification of Functioning, Disability, and Health. Reproduced with permission from [6].

Musculoskeletal outcome: the body—assessment of structure and function

Until recently, most long-term musculoskeletal outcome studies have used clinical and radiologic scores, in addition to the number and frequency of bleeding episodes, to determine the efficacy of different treatment regimes [7–10]. With the advent of early prophylaxis, the incidence of joint arthropathy has significantly reduced [7–10]. As the aim of therapy changed from preventing severe joint damage to maintenance of a normal joint with prophylactic therapy, it became necessary

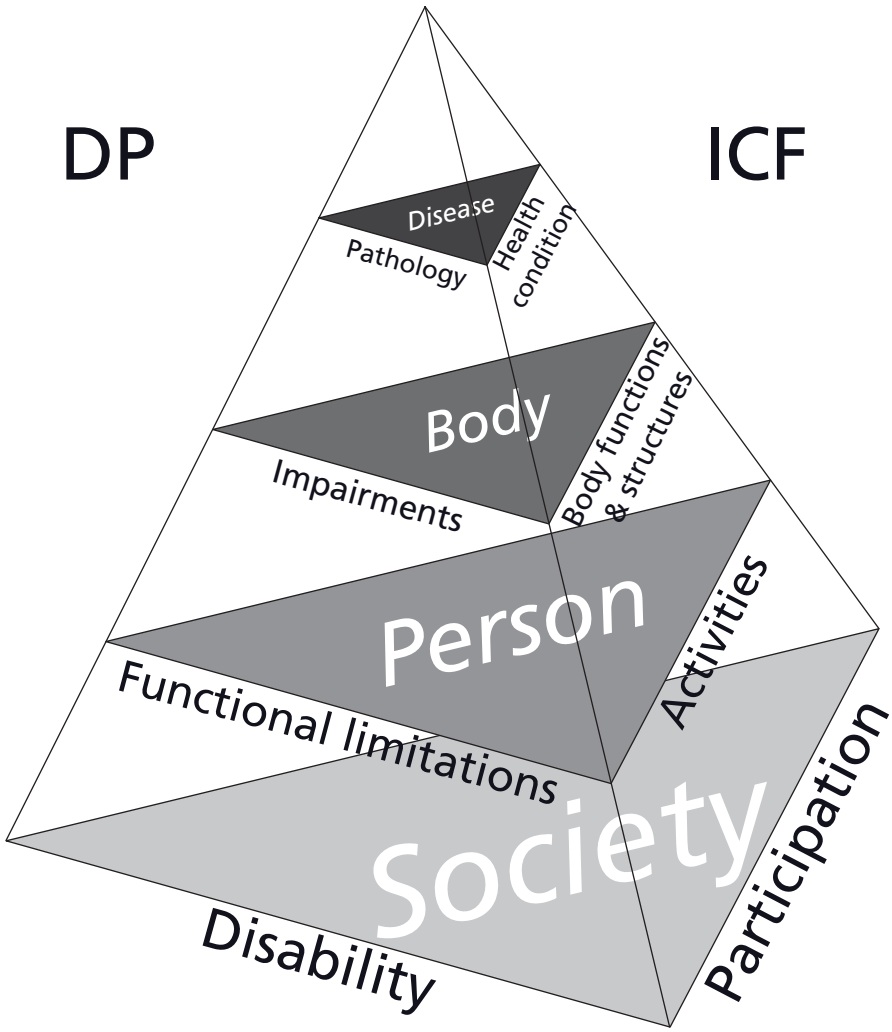

Figure 32.2 Parallels between the Disablement Process (DP) and the International Classification of Functioning, Disability, and Health (ICF).

Table 32.1 Physical joint examination score endorsed by the Orthopaedic Advisory Committee of the World Federation of Hemophilia (WFH) [3].

Physical finding	Score	Scoring key
Swelling	0 or 2+ (S)	0 = none 2 = present S if chronic synovitis present
Muscle atrophy	0–1	0 = ≤1 cm 1 = present
Axial deformity (measured at knee and ankle only)		
Knee	0–2	0 = 0–7° valgus 1 = 8–15° valgus or 0–5° varus 2 = >15° valgus or >5° varus
Ankle	0–2	0 = no deformity 1 = ≤10° valgus or ≤5° varus 2 = >10° valgus or >5° varus
Crepitus on motion	0–1	0 = none 1 = present
Range of motion	0–2	0 = loss of <10% of total FROM (full range of movement) 1 = loss of 10–33% of total FROM 2 = loss of >33% of total FROM
Fixed contracture	0 or 2	0 = <15% fixed flexion contracture 2 = ≥15% fixed flexion contracture at hip and knee and equinus at ankle
Instability	0–2	0 = none 1 = present, but neither interferes with function nor requires bracing 2 = instability that creates a functional deficit or requires bracing.
Total	0–12 0–10	Ankle or knee Elbow

to improve these tools, and develop new ones, to make them more sensitive to early changes.

Physical examination scores

Physical joint assessment is commonly used in literature to measure structural and functional joint damage, as it is readily available and inexpensive. ROM has been the most commonly used physical outcome measure for evaluating the effects of intervention on joint health [11,12]. As more medical and surgical methods to improve function became available, it became necessary to develop an instrument that could assess a wider spectrum of physical changes that occur as a result of joint damage. With this in mind, the WFH endorsed the physical examination (PE) scale (Table 32.1). This scoring system, first described in 1985 [3,13], evaluates ROM, flexion deformity, swelling, crepitus, muscle wasting, instability, and axial deformity in the six major joints (i.e., ankles, knees, and elbows). A score of zero denotes a normal joint. The ankle and knees can score as high as 12 points, and the elbows a maximum of 10—giving a total of 68 for the six joints. Each joint is also scored for pain (0–3), and in some studies this has been added to the total score (Table 32.2). However, this scale was adopted and recommended for routine use at a time when most hemophilia patients in Western countries used much lower quantities of replacement therapy than they do now. In the Orthopaedic Outcome Study, reported in 1994, 90% of patients with hemophilia demonstrated changes in their joints, as assessed by radiologic and physical examination scores [9]. As larger quantities of factor concentrates were used for replacement therapy, and as the frequency of bleeding was reduced with the use of primary prophylaxis, more joints

Table 32.2 Pain instrument recommended by the Orthopaedic Advisory Committee of the World Federation of Haemophilia (WFH).

	Score	Scoring key
Pain	0–3	0 = no pain, no functional deficit, no analgesic use except with acute hemarthrosis 1 = mild pain, does not interfere with occupation nor with activities of daily living; may require occasional non-narcotic analgesics 2 = moderate pain, partial or occasional interference with occupation or activities of daily living 3 = severe pain. Interferes with occupation or activities of daily living, requires frequent use of non-narcotic and narcotic medications

maintained zero (normal) scores on the WFH PE scale [14]. In addition to this lack of sensitivity, the original WFH score has several other shortcomings. For instance, it does not take into account the normal physiologic changes that occur in children (i.e., changes in hyperextensibility, axial deformity, and muscle bulk differences between dominant and nondominant limbs) [15]. Furthermore, the score does not assess strength—an important function that affects physical activity.

These observations provided the impetus to develop new scoring systems with improved sensitivity and the ability to detect earlier deterioration in joint function. One such system was the Colorado PE instruments (full and half point), described by Manco-Johnson *et al.* [15]. Development of this scale involved modification of the WFH PE score (addition of one grade) to make it more sensitive in the areas of swelling, muscle atrophy, crepitus, ROM, and flexion contracture. Evaluations of gait and strength were also added, with strength being assessed using functional (rather than confrontational) tests. The same group also described the Young Child Scale [15], in which pain is assessed using the Wong Baker Faces Pain Scale. The PedNet group also introduced another tool based on the WFH PE score (the Stockholm instrument) in an attempt to increase the sensitivity of physical assessment [16].

Subsequently, these scores were combined by the International Prophylaxis Group (IPSG) to produce the Hemophilia Joint Health Score (HJHS) (Table 32.3). The aim was to produce a score that would be sensitive to early change, account for normal development in children, and be reliable, valid, and practical to administer. The initial validity was assessed in eight pediatric patients, and the interobserver coefficient (as assessed by scoring the patients on two consecutive days) was found to be 0.83, and the reliability of the test was found to be excellent [17]. Following a multicenter validation study, a second version was developed by removing or modifying redundant or less sensitive items (P. Hilliard, The Hospital for Sick Childre, Toronto, Canada, personal communication). The score for joint pain has been altered (to a score of either 0, 1, or 2), and the score for axial alignment, joint instability and gait have been removed, giving a total possible score of 20 for each joint, in addition to a maximum score of 4 for assessment of global gait. The HJHS will need further evaluation in other patient populations and in other centers not involved in its design to be able to assess its applicability and usefulness in patient care and research.

Table 32.3 Functional Independence Score in Haemophilia (FISH) [34].

Self-care	Transfers	Locomotion
Eating and grooming	Chair	Walking
Bathing	Squatting	Step climbing
Dressing		Running

Score from 1–4 in each area:

4 The subject is able to perform the activity without any difficulty like other healthy peers.

3 The subject is able to perform the activity without aids or assistance, but with slight discomfort. He is unable to perform the activity like his healthy peers.

2 The subject needs partial assistance/aids/modified instruments/modified environment to perform the activity.

1 The subject is unable to perform the activity, or needs complete assistance to perform the activity.

Radiologic scores

De Palma, in 1956, and Jordon, in 1958, described the characteristic X-ray abnormalities seen in hemophilic arthropathy [18]. De Palma proposed four classes of joint destruction based on a combination of roentgenographic and clinical manifestations [18]. In 1977, Arnold and Hilgartner refined this classification into five stages [19]. Wood proposed a radiologic classification in 1969, but several parameters he described pertained to soft-tissue changes seen in the acute stage of hemarthrosis [20]. In 1980, Pettersson created a scoring system based on the degree of joint destruction, as assessed by radiologic changes seen in the six most commonly affected joints (i.e., the knee, elbow, and ankle joints) [2]. This was incorporated into the first joint-scoring system endorsed by the World Federation of Hemophilia (WFH) [2,3,13]—a system that has so far been the standard for long-term outcome measurement in hemophilia (Table 32.4).

Table 32.4 World Federation of Haemophilia Pettersson scale of hemophilic arthropathy.

Type of change	Finding	Score
Osteoporosis	Absent	0
	Present	1
Enlarged epiphysis	Absent	0
	Present	1
Irregular subchondral surface	Absent	0
	Partially involved	1
	Totally involved	2
Narrowing of joint space	Absent	0
	Joint space >1 mm	1
	Joint space <1 mm	2
Subchondral cyst formation	Absent	0
	One cyst	1
	More than one cyst	2
Erosion of joint margins	Absent	0
	Present	1
Gross incongruence of articular bone ends	Absent	0
	Slight	1
	Pronounced	2
Joint deformity (angulation and/ or displacement between articulating bones)	Absent	0
	Slight	1
	Pronounced	2
Possible joint score		**0–13**

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The introduction of more intensive prophylaxis in younger children enabled joints to be maintained at a stage where no radiologic changes could be detected [7,8]. As more patients maintained scores of 0 (normal joints), it became evident that conventional radiographs were insensitive to early changes in the hemophilic joint. Consequently, scoring systems based on magnetic resonance imaging (MRI) were developed. These could detect changes to the joint before they were apparent in X-rays. Changes observed by MRI were first described in 1986 by Kulkarni *et al.* [21], and several other reports of MRI use soon followed. The Denver scale [22], the European scale used by the Pediatric Network for Hemophilia Management (PedNet) [23], and the scale currently being developed by the International Prophylaxis Study Group (IPSG) [24] are all examples of scores based on MRI changes. These scoring systems will be important in assessing minimal joint arthropathy. However, the implications of minor changes in MRI/radiologic scores in terms of individual joint function and overall long-term musculoskeletal functional ability remain to be determined. Further, the cost and time involved in measuring the six major joints will make it difficult to be accepted as a standard assessment tool in long-term outcome studies in most centers around the world.

Other alternatives, such as the ultrasonographic evaluation of the soft-tissue changes in hemophilic arthropathy, are currently under study [25]. The significance of the soft-tissue changes seen in the hemophilic joints need to be correlated with the severity of the disease, and the clinical significance of the various changes need to be assessed. Its role in detecting early joint damage needs to be assessed. Ultrasonographic examination, along with plain radiography, may offer additional information, especially in settings where MRI is not available.

Musculoskeletal outcome: the person—assessment of activities and functional independence in hemophilia

Until recently, assessment of musculoskeletal function in hemophilia relied predominately on the clinical and radiologic measurement of structural changes in individual joints. There are several limitations with this approach. For those on prophylaxis with different doses of factors, the clinical significance of small differences noted in these scores was unclear. Also, for those on different levels of on-demand replacement therapy, where there is greater joint damage, the clinical significance of differences in the scores is not clear [4,26]. Taking into account both these situations, it became essential to develop instruments that would assess other areas of musculoskeletal function as well. Several validated outcome instruments are available to assess functional independence in patients with other arthritic conditions [27–29]. Some of these measure patients' general health status, while others measure outcomes specific to an organ or disease condition. Several of the instruments have been used to assess patients with hemophilia. The Short Form of the Medical Outcomes Study (SF-36) [27] and the Sickness Impact Profile (SIP) [28] do not refer to any specific disease, but provide information relating to general health. The Western Ontario McMaster Questionnaire (WOMAC) [29] was originally designed to be used in patients with osteoarthritis, while the Stanford Health Assessment Questionnaire (HAQ) [30] and the Childhood Health Assessment Questionnaire (CHAQ) [14] are used primarily for patients with juvenile rheumatoid arthritis. De Kleijn *et al.* identified 34 clinimetric instruments that have been used to assess the functional health status in persons with hemophilia [31]. Thirty-three instruments were classified according to the ICF, of which 17 measured body function and structure, 12 measured activities, and four measured participation. Of those that measured activities, none were validated for use in hemophilia, and none were performance based. They concluded that assessment of functional health in persons with hemophilia needs further development. In another review of 40 clinimetric instruments used in rheumatoid arthritis and osteoarthritis, it was found that only four instruments (EPM-ROM, AIMS-2, SF-36, and WOMAC) had been assessed fully for their psychometric properties [1].

In 2004, Van Genderen *et al.* started developing the Haemophilia Activities List (HAL), a hemophilia-specific self-assessment questionnaire to assess functional abilities in hemophilia [32]. The final version was subsequently validated in 2006 [33]. Initially, over 150 adult patients with severe hemophilia were interviewed and asked for their main problematic activities in daily life. These activities served as items for the questionnaire. Subsequently, several focus groups commented on the HAL and adjustments were made to its content [32]. Further research, focusing on statistical analyses, finally resulted in a 42-item questionnaire with good convergent validity (r = 0.47–0.84) [33]. The construct validity of the HAL when compared with four performance-based tests was generally lower (r = 0.23–0.77), as was expected [33]. The internal consistency of the HAL was also found to be good to very good (Cronbach's α = 0.61–0.97) [33].

The HAL can be administered in approximately 5 min. It consists of 42 activity items, divided among seven domains: "lying down/sitting/kneeling/standing," "functions of the legs," "functions of the arms," "use of transportation," "self care," "household tasks," and "leisure activities and sports". Scores can be obtained for each domain separately, but an overall sum score for the complete questionnaire can also be calculated. Additionally, three component scores are also available in order to quantitate functional abilities relating to upper extremity functioning and basic and complex lower extremity functioning. Scores can range from 0 (indicating the worst possible situation) to 100 points (indicating the best possible situation) [32,33].

A possible pitfall when using self-report questionnaires is that such instruments are language and culture specific. In a cohort of patients from India, it was found that several

questions, such as those relating to household tasks and leisure, were not attempted by most patients; only 10% of the patients completed all 42 items [34]. The HAL, however, does allow for unanswered questions, since the normalization procedure when calculating the final scores adjusts for missing items. In addition, a large number of the patients evaluated in the Indian cohort were children, and it is to be noted that the HAL was developed in close collaboration with, and validated for use in, adults (mean age of 45 years) [32,33]. Currently, a pediatric version of the HAL (called PedHAL) is being developed.

When using any self-reported instrument such as the HAL, there are certain steps to be considered. If the questionnaire is translated, that translated version needs to be validated before use. In countries where literacy is often a major issue, interview-based assessment tools are often preferred over self-rated tools to assess subjective improvement in function. However, a questionnaire that is designed as a self-rated instrument should not be scored using an interview method, unless the score has been validated as an interview-based tool.

In addition to the self-reported HAL, the Functional Independence Score in Haemophilia (FISH) was developed as a performance-based assessment tool to objectively measure the patient's functional ability [34].The final assessment included eight activities (eating, grooming, dressing, chair transfer, squatting, walking, step climbing, and running) that were graded from 1 to 4 according to the amount of assistance required to perform them (Table 32.3). Each activity and level of independence was clearly defined to reduce inter-observer variance. It has good internal consistency (Cronbach's $\alpha = 0.85$), indicating that all parts of the instrument contributed significantly to the final score. Activities such as squatting and running were found to have greater discriminatory value, with larger number of individuals not being able to perform these activities. Therefore, even though squatting may not be a frequently required functional activity all over the world, it is a useful part of this instrument, and could be of particular value in those with apparently less knee joint damage [34].

The FISH was found to be highly reliable, with a pooled intraclass correlation coefficient of 0.98. It has good construct validity, with a good correlation with other self-rated assessment tools, including the HAL. The FISH had a good correlation with the clinical score ($r = -0.61$). The radiologic score, however, had only a moderate correlation with functional disability ($r = -0.38$) [34]. Many patients with hemophilia appear to function reasonably well despite having poor X-ray scores. This has been found to be true in osteoarthritis as well [35]. This emphasizes the limitation of using radiologic scores alone in assessing musculoskeletal outcome of care in hemophilia.

The FISH, being objective in nature, requires a therapist, nurse, or physician to assess the patient's functional ability. In addition, it may not always reflect the patient's functional priorities. Another limitation of the FISH is that it does not have provisions for assessment of improvement in function of "normal" individuals following interventions like sports training or exercise programs. FISH was originally designed to compare the patient's basic functional ability with that of normal healthy individuals. Improvement in function of these "normal" individuals could potentially be measured using other objective tests like the 6-min walk test or energy consumption assessment. Other tests, such as the 50-meter walking test, timed-up-and-go test, and the figure-of-eight walking test have been used in other diseases to objectively assess function, and could be incorporated into FISH to make it more useful for patients with minimal joint changes [30].

While it is important to assess the patient's perception of his functional ability using self-rated instruments, it is also necessary to objectively quantitate how the individual is able to perform. In performance-based instruments, strength, joint mobility, and other physical traits are the main performance-limiting factors, as opposed to the patient's beliefs or perceptions [36]. It is known from the literature that both types of instruments measure different constructs of physical functioning, and the two types of scores complement each other [36–38]. Following treatment, the subjective score gives us an impression of the patient's perception of improvement, whereas the performance-based score gives us objective information on which aspects of function have changed to give the individual an impression of improvement. We therefore recommend using both self-reported and performance-based instruments. In assessment of functional abilities in adult hemophiliacs, the combination of the HAL and the FISH seems the most appropriate.

Musculoskeletal outcome: in society—assessment of participation and quality of life

Although the above-mentioned clinimetric tools provide a measure of the patient's ability to perform activities, it does not study the effect of hemarthrosis on a patient's ability to participate in social activities. Days missed from work, and the ability to engage in sports/physical activities, are not assessed. These activities affect QoL and are measured using other scoring systems such as the SF36 [27], the Arthritis Impact Measurement Scale (AIMS) [39], and the EuroQoL Five-Dimension Questionnaire (EQ-5D) [40]. More recently, hemophilia-specific scores, such as the Haemo-QoL [41], the Canadian Health Outcomes-Kids Life Assessment Tool (CHO-KLAT) [42], the Hemofilia-QoL [43], and the Hemolatin-QoL [44], have been developed and studied in the different populations. The usefulness of these tools in the management of persons with hemophilia will need to be evaluated in a range of socioeconomic and cultural situations before any of them can be accepted as a universal assessment tool in hemophilia. As QoL questionnaires are cumulative outcome measures, giving little insight into the problems that give rise to a dimin-

ished QoL score, they may not be helpful in targeting interventions [32]. Evaluation of QoL is often difficult to interpret, as there are several independent factors that affect the measured outcome—such as availability of basic amenities like food, clothing, and shelter, in addition to availability of treatment and employment. The assessment may not be a reflection of the effect of interventions for hemophilia alone. Moreover, even in affluent societies, QoL could potentially be affected by the individual's acceptance of the fact that hemophilia is "integral to the self," and finding a niche where the individual could be successful [45].

Conclusion

The predominant cause of morbidity in hemophilia is the damage resulting from repeated bleeding into joints. It has therefore been the aim of therapy to establish a standard where there is no evidence of damage to the joints, both clinically and radiologically. The musculoskeletal outcome of a person with severe hemophilia was perhaps not very different in varying parts of the world until the 1960s. However, in the last four decades, the ability of factor replacement therapy to completely modify the clinical outcome of severe hemophilia has been recognized. In most Western countries, treatment for hemophilia has focused on preventing joint arthropathy by administering a high dose of factor replacement [7–10,46]. With good access to factors, it has been possible for these countries to reduce the morbidity associated with hemophilia [46]. A person with hemophilia can now lead a nearly normal life without fear of frequent bleeding into joints [14]. The effectiveness of this approach in preserving joint integrity has been so good that it has now become the standard for therapy in economically developed countries. Unfortunately, the annual cost of such therapy, at US$100 000–300 000 per person, has been so high that it has been difficult for all countries to adopt it universally [47].

While it is desirable for people with hemophilia all over the world to have the same level of care, the reality is that it is not feasible economically. The aim of clotting factor replacement therapy in such conditions shifts from maintaining normal joints to good overall joint function that will allow the person to remain independent in his activities until more intensive factor replacement therapy becomes possible [4,26]. To develop optimal protocols for management—whether it is prophylaxis at high or moderate doses [46] or on-demand therapy at different doses—it is important that adequate data on outcome be documented. Such data can be most useful, not only for health authorities, but also for patients, to better understand their treatment plans and likely outcome. There is great interest in developing clinimetric instruments for this purpose. The challenge is in making them universally applicable and then encouraging care providers and patients to use them for documenting long-term outcome. A systematic evaluation of musculoskeletal outcome using different clinimetric instruments will help develop suitable models of treatment of hemophilia, and will also assist healthcare planners in determining the cost of care.

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References

1 De Kleijn P, Van Genderen FR, Van Meeteren NLU. Assessing functional health status in adults with haemophilia: Towards a preliminary core set of clinimetric instruments based on a literature search in Rheumatoid Arthritis and Osteoarthritis. *Haemophilia* 2005; **11**: 308–18.
2 Pettersson H, Ahlberg A, Nilsson IM. A radiologic classification of hemophilic arthropathy. *Clin Orthop Relat Res* 1980; **149**: 153–9.
3 Pettersson H, Gilbert MS. Classification of the hemophilic arthropathy. In: Pettersson H, Gilbert MS (eds.) *Diagnostic Imaging in Hemophilia*. New York: Springer-Verlag, 1985: 56–68.
4 Poonnoose PM, Srivastava A. Functional assessment of haemophilic arthropathy—An international perspective. *Semin Hematol* 2006; **43**(Suppl. 1): S27–S32.
5 Verbrugge LM, Jette AM. The disablement process. *Soc Sci Med* 1994; **38**: 1–14.
6 World Health Organization. *International Classification of Functioning, Disability and Health (ICF)*. Geneva: World Health Organization, 2001.
7 Lofqvist T, Nilsson IM, Berntorp E, Pettersson H. Haemophilia prophylaxis in young patients—a long-term follow-up. *J Intern Med* 1997; **241**: 395–400.
8 Fischer K, van der Bom JG, Molho P, *et al*. Prophylactic versus on-demand treatment strategies for severe haemophilia: A comparison of costs and long-term outcome. *Haemophilia* 2002; **8**: 745–52.
9 Aledort LM, Haschmeyer RH, Pettersson H, and the Orthopaedic Outcome Study Group. A longitudinal study of orthopaedic outcomes for severe factor-VIII-deficient haemophiliacs. *J Intern Med* 1994; **236**: 391–9.
10 Nilsson IM, Berntorp E, Lofqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. *J Intern Med* 1992; **232**: 25–32.
11 Ekert H, Moorehead M, Williamson G. Home treatment of haemophilia. A follow-up study. *Med J Aust* 1981; **2**: 21–3.
12 Rodriguez-Merchan EC. Therapeutic options in the management of articular contractures in haemophiliacs. *Haemophilia* 1999; **5**(Suppl. 1): 5–9.
13 Rodriguez-Merchan EC, de la Corte H. Musculoskeletal outcomes research in haemophilia. In: Rodriguez-Merchan EC (ed.) *The Haemophilic Joints. New Perspectives*. Oxford: Blackwell Publishing, 2003: 36–44.
14 Schoenmakers MAGC, Gulmans VAM, Helders PJM, Van Den Berg HM. Motor performance and disability in Dutch children with haemophilia: A comparison with their healthy peers. *Haemophilia* 2001; **7**: 293–8.

15 Manco-Johnson MJ, Funk S. Joint evaluation instruments in haemophilia. In: Rodriguez-Merchan EC (ed.) *The Haemophilic Joints. New Perspectives*. Oxford: Blackwell Publishing, 2003: 45–55.
16 Hill F, Ljung R; European Paediatric Network for Haemophilia Management. Third and fourth workshops of the European Paediatric Network for Haemophilia Management. *Haemophilia* 2003; **9**: 223–8.
17 Hilliard P, Funk S, Zourikian N, *et al*. Hemophilia joint health score reliability study. *Haemophilia* 2006; **12**: 518–25.
18 DePalma AF. Hemophilic arthropathy. *Clin Orthop Relat Res* 1967; **52**: 145–65.
19 Arnold WD, Hilgartner MW. Haemophilic arthropathy. Current concepts of pathogenesis and management. *J Bone Joint Surg Am* 1977; **59**: 287–305.
20 Wood K, Omer A, Shaw MT. Haemophilic arthropathy. A combined radiological and clinical study. *Br J Radiol* 1969; **42**: 498–505.
21 Kulkarni MV, Drolshagen LF, Kaye JJ, *et al*. MR imaging of hemophilic arthropathy. *J Comput Assist Tomogr* 1986; **10**: 445–9.
22 Nuss R, Kilcoyne RF, Geraghty S, *et al*. MRI findings in haemophilic joints treated with radiosynoviorthesis with development of an MRI scale of joint damage. *Haemophilia* 2000; **6**: 162–9.
23 Lundin B, Pettersson H, Ljung R. A new magnetic resonance imaging scoring method for assessment of haemophilic arthropathy. *Haemophilia* 2004; **10**: 383–9.
24 Doria AS, Babyn PS, Lundin B, *et al*. Reliability and construct validity of the compatible MRI scoring system for evaluation of haemophilic knees and ankles of haemophilic children. Expert MRI working group of the international prophylaxis study group. *Haemophilia* 2006; **12**: 503–13.
25 Zukotynski K, Jarrin J, Babyn PS, *et al*. Sonography for assessment of haemophilic arthroplasty in children: A systematic protocol. *Haemophilia* 2007; **13**: 293–304.
26 Fischer K, Van Den Berg HM, Thomas R, *et al*. Dose and outcome of care in haemophilia—How do we define cost-effectiveness? *Haemophilia* 2004; **10**(Suppl. 4): 216–20.
27 Fischer K, Bom JG, Mauser-Bunschoten EP, Roosendal G, Berg HM. Effects of haemophilic arthropathy on health-related quality of life and socio-economic parameters. *Haemophilia* 2005; **11**: 43–8.
28 Bergner M, Bobbitt RA, Kressel S, Pollard WE, Gilson BS, Morris JR. The sickness impact profile: Conceptual formulation and methodology for the development of a health status measure. *Int J Health Serv* 1976; **6**: 393–415.
29 Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: A health status instrument for measuring clinically important patient-relevant outcomes to antirheumatic therapy in patients with osteoarthritis of the hip or knee. *J Rheumatol* 1988; **15**: 1833–40.
30 Solovieva S. Clinical severity of disease, functional disability and health-related quality of life. Three-year follow-up study of 150 Finnish patients with coagulation disorders. *Haemophilia* 2001; **7**: 53–63.
31 De Kleijn P, Heijnen L, Van Meeteren NLU. Clinimetric instruments to assess functional health status in patients with haemophilia: A literature review. *Haemophilia* 2002; **8**: 419–27.
32 Van Genderen FR, Van Meeteren NLU, Van Der Bom JG, *et al*. Functional consequences of haemophilia in adults: The development of the Haemophilia Activities List. *Haemophilia* 2004; **10**: 565–71.
33 Van Genderen FR, Westers P, Heijnen L, *et al*. Measuring patients perceptions on their functional abilities: Validation of the Haemophilia Activities List. *Haemophilia* 2006; **12**: 36–46.
34 Poonnoose PM, Thomas R, Keshava SN, *et al*. Psychometric analysis of the Functional Independence Score in Haemophilia (FISH). *Haemophilia* 2007; **13**: 620–6.
35 Bruyere O, Honore A, Rovati LC, *et al*. Radiologic features poorly predict clinical outcomes in knee osteoarthritis. *Scand J Rhuematol* 2002; **31**: 13–16.
36 Wittink H, Rogers W, Sukiennik A, Carr DB. Physical functioning: Self-report and performance measures are related but distinct. *Spine* 2003; **28**: 2407–13.
37 Isernhagen SJ. Functional capacity evaluation: Rationale, procedure, utility of the kinesiophysical approach. *J Occup Rehab* 1992; **2**: 157–68.
38 Hoeymans N, Feskens EJ, Van den Bos GA, Kromhout D. Measuring functional status: Cross-sectional and longitudinal associations between performance and self-report (Zutphen Elderly Study 1990–1993). *J Clin Epidemiol* 1996; **49**: 1103–10.
39 Tanaka S, Hachisuka K, Okazaki T, Shirahata A, Ogata H. Health status and satisfaction of asymptomatic HIV-positive haemophiliacs in Kyushu, Japan. *Haemophilia* 1999; **5**: 56–62.
40 Trippoli S, Vaiani M, Linari S, Longo G, Morfini M, Messori A. Multivariate analysis of factors influencing quality of life and utility in patients with haemophilia. *Haematologica* 2001; **86**: 722–8.
41 von Mackensen S, Bullinger M; Haemo-QoL Group. Development and testing of an instrument to assess the Quality of Life of Children with Haemophilia in Europe (Haemo-QoL). *Haemophilia* 2004; **10**(Suppl. 1): 17–25.
42 Young NL, Bradley CS, Blanchette V, *et al*. Development of a health-related quality of life measure for boys with haemophilia: The Canadian Haemophilia Outcomes—Kids Life Assessment Tool (CHO-KLAT). *Haemophilia* 2004; **10**(Suppl. 1): 34–43.
43 Arranz P, Remor E, Quintana M, *et al*. Development of a new disease-specific quality-of-life questionnaire to adults living with haemophilia. *Haemophilia* 2004; **10**: 376–82.
44 Remor E, Young NL, von Mackensen S, Lopatina EG. Disease-specific quality-of-life measurement tools for haemophilia patients. *Haemophilia* 2004; **10** (Suppl. 4): 30–34.
45 Beeton K, Neal D, Lee C. An exploration of health related quality of life in adults with haemophilia—A qualitative perspective. *Haemophilia* 2005; **11**: 123–32.
46 Fischer K, Astermark J, Van der Bom JG, *et al*. Prophylactic treatment for severe haemophilia: Comparison of an intermediate-dose to a high-dose regimen. *Haemophilia* 2003; **8**: 753–60.
47 Manco-Johnson MK, Abshire TC, Shapiro AD, *et al*. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe haemophilia. *N Engl J Med* 2007; **357**: 535–44.

33 Hepatitis and hemophilia

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Introduction

The introduction of clotting factor concentrates in the late 1960s and 1970s revolutionized the lives of hemophiliacs, reducing the morbidity and mortality associated with their disease. This improvement was so marked that it was estimated that in the late 1970s the life expectancy of hemophiliacs treated with concentrate approached that of the normal population. Unfortunately, this optimism was premature and the development of serious infections in the form of viral hepatitis and human immunodeficiency virus (HIV) led to new problems affecting the lives of hemophiliacs. Table 33.1 lists the infections transmitted by concentrates, their physical characteristics, and the ability of viral inactivation processes to destroy them.

Clotting factor concentrates are prepared from plasma pools of up to 30 000 donations and prior to viral inactivation any infections present in the plasma donors could be transmitted to the recipient. Table 33.2 shows the prevalence of concentrate-transmitted infections in Sheffield patients treated with nonvirally inactivated concentrates prior to 1985.

The introduction of viral inactivation in 1985 largely eliminated the risk of hepatitis virus transmission by concentrates. Although some of the early viral inactivation methods were not 100% successful, there have been no hepatitis transmissions by concentrates since the early 1990s. All plasma-derived products in Europe now have to undergo viral inactivation by two different processes [1]. Recombinantly engineered products are free from the risk of hepatitis virus transmission.

Hepatitis viruses in hemophilia

Hepatitis A virus (HAV)

Acute hepatitis A infection can be demonstrated by the detection of IgM anti-HAV antibodies. Paired serum testing is not required. HAV RNA testing in serum, stool, or liver by polymerase chain reaction (PCR) is a research tool. Past infection is demonstrated by the detection of IgG anti-HAV.

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As a result of donor screening, plasma pool testing, viral inactivation of concentrates, and vaccination of recipients, the risk of transmission of this virus by concentrates has been eliminated. In the early 1990s there were a number of outbreaks of HAV in hemophiliacs treated exclusively with solvent/detergent (S/D) [2]. This was not so surprising because S/D does not inactivate viruses without a lipid envelope, such as HAV.

Hepatitis B virus (HBV)

Hepatitis B virus transmission was common prior to viral inactivation of concentrates with 50% of patients infected. Most adult patients cleared the virus spontaneously and in countries where hepatitis B is not endemic, only 2–5% of hemophiliacs are now chronically infected with hepatitis B. Because patients with bleeding disorders may receive non- or poorly virally inactivated blood products whilst visiting other (less developed) countries, it is recommended that all patients with inherited bleeding disorders should be appropriately vaccinated against hepatitis B and hepatitis A.

Hepatitis C virus (HCV)

Hepatitis C infection occurred in virtually all recipients of clotting factor concentrates at their first exposure [3]. Past exposure is indicated by the identification of anti-HCV antibodies while current infection is demonstrated by the detection of both anti-HCV and HCV RNA by PCR. All HCV RNA-positive patients should have their HCV genotype established. A total of 80–90% of hemophiliacs in Europe are infected with genotype 1, 10–15% with genotype 2 or 3, and <5% with genotypes 4, 5, or 6 [4]. Surprisingly, despite the repeated infusion of infected concentrates to hemophiliacs prior to 1995, mixed infections are rare but this may be because of a methodological problem where the PCR detects only the dominant genotype.

Monitoring of chronic hepatitis C

Chronic HCV is generally a relatively slowly progressive disease and its rate of progression varies significantly between

Table 33.1 The hepatitis viruses that can be transmitted by blood products, and their characteristics.

Virus	Genome	Lipid envelope	Size (nm)	Solvent/ detergent resistant	Heat resistant
Hepatitis A	RNA	No	27	Yes	No
Hepatitis B	DNA	Yes	42	No	No
Hepatitis C	RNA	Yes	35–65	No	No
Hepatitis D	RNA	Yes	35	No	No

Table 33.2 Prevalence of viral infections in patients at the Sheffield Haemophilia and Thrombosis Centre treated with nonvirally inactivated concentrates.

Viral infection	% positive
Previous hepatitis A	37
Current hepatitis B	2
Previous hepatitis B	48
Hepatitis C	100
HIV	26

Table 33.3 Definitions used in the assessment of hepatitis C treatment.

Chronic hepatitis C virus (HCV)—Persisting infection (HCV RNA positive) 6 months or more after acute infection
Rapid virological response (RVR)—Negative HCV RNA at 4 weeks
Partial early virological response (pEVR)—Reduction of HCV RNA load by >2 logs (i.e., <1% level) 12 weeks after starting treatment
Complete early virological response (cEVR)—Negative HCV RNA at 12 weeks
End of treatment response (ETR)—HCV RNA negative at treatment end
Sustained virological response (SVR)—HCV RNA negative 6 months after the end of treatment
Transient response—Reduction in HCV RNA by >2 logs during treatment but remaining positive at end of treatment
Relapse—HCV RNA negative at end of treatment but relapse after this
Nonresponse—No reduction of HCV RNA by >2 logs at any point during treatment

individuals. Monitoring is required to identify individuals with advanced disease. Monitoring methods can be subdivided into biochemical, radiologic, and histologic.

Biochemical monitoring

The most common method for monitoring the activity of HCV infection is via estimation of the serum aminotransferases [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)], which can reflect inflammatory activity in the liver. It is also common to routinely measure serum albumin, bilirubin, and prothrombin time, but these do not become abnormal until there is advanced liver disease. The problem with ALT and AST estimation is that they poorly reflect the severity of liver disease and can be entirely normal in a proportion of patients with cirrhosis [5]. The upper limit of normal requires reappraisal.

Recently, a number of algorithms based on biochemical testing have been introduced, which claim to more accurately predict the severity of the liver disease. In one of these, the Fibrotest, the following are used in a patented algorithm to derive a score correlating liver disease severity: age, sex, α_2-macroglobulin, haptoglobin, γ-GT, total bilirubin, and apolipoprotein A1. In a study of hemophiliacs from Israel, Maor and colleagues used the Fibrotest in 132 hemophiliacs [6]. They were able to correctly identify the differing stage in patients who have cleared the virus and those with known advanced disease.

Hepatic imaging

The most commonly performed radiologic or imaging technique to evaluate hepatic fibrosis, architecture, or hepatic masses is ultrasound examination of the liver. Computed tomography (CT) and magnetic resonance imaging (MRI) may be used. The radiologic tests are quite sensitive for detecting hepatocellular carcinoma larger than 1 cm in size but are relatively poor at documenting liver fibrosis unless this is very advanced and portal hypertension is present.

A recent development has been the introduction of the transient elastography, or Fibroscan, which uses a probe to measure propagation of an ultrasound shear wave and hence measures the elasticity of the liver. In a study in hemophiliacs, Posthouwer and colleagues [7] found that 18% of 124 unselected hemophiliacs with chronic HCV had severe fibrosis and 17% had cirrhosis. Only 7% of patients were previously known to have cirrhosis by the other standard tests.

Histologic monitoring

Direct microscopic examination of liver tissue remains the gold standard in establishing disease stage, inflammation, and severity. Understandably, there is a reluctance to perform biopsies in patients with inherited bleeding disorders. In patients without hemophilia, the risk of any bleeding during liver biopsy is 1 in 1000 and the risk of fatal bleeding is 1 in 10 000 [8]. Following two deaths of hemophiliacs during liver biopsies in the early 1980s in London and New York, it was suggested that the risk of bleeding in hemophilia is much higher than in other patients. The precise risk of bleeding in hemophilia has not been accurately defined. In Sheffield we have performed over 100 liver biopsies with only one episode

of mild bleeding (hemobilia) treated with factor VIII (FVIII) concentrate alone [9]. Liver biopsy can be performed through the transcutaneous or preferably via the transjugular routes. Irrespective of the method used, biopsies should ideally be done under direct radiologic imaging.

Patients with hemophilia should have their bleeding disorder normalized with infused concentrate or desmopressin prior to liver biopsy. A number of protocols exist and the one used in Sheffield consists of:

- immediately prebiopsy—treat to 1.00 IU/ml, i.e., 100%
- 12 h post biopsy—measure level and treat to 1.00 IU/ml
- 24 h post biopsy—measure level and treat to 1.00 IU/ml
- 48 h post biopsy—measure level and treat to 1.00 IU/ml.

The fact that liver biopsies can now be performed in hemophiliacs relatively safely does not mean that liver biopsy is mandatory. Most patients can be treated for chronic hepatitis C with pegylated interferon and ribavirin irrespective of their liver histology. Biopsies remain useful for patients with normal transaminases who do not wish to receive anti-HCV therapy to establish that their hepatitis is mild, or where the cause of the liver dysfunction is in doubt, or where cirrhosis needs to be established before transplantation or for surveillance for hepatocellular carcinoma (HCC).

Natural history of hepatitis C

Following acute HCV infection the disease leads to chronic infection (defined as HCV RNA positivity >6 months after acute infection) in 80–85%. Although deaths during acute infection have been reported in nonhemophiliacs, this is very rare, occurring in <1 in 10 000. Patients with chronic HCV seldom clear the virus spontaneously, but spontaneous resolution has been reported. Patients with active chronic hepatitis C may develop cirrhosis, decompensated cirrhosis, end-stage liver failure, or HCC (Figure 33.1).

Spontaneous clearance

A total of 15–20% of patients infected with hepatitis C clear the virus spontaneously during the acute phase [10]. This is more likely to occur when patients are infected in childhood. Once chronic hepatitis C is established 6 months after infection, spontaneous clearance rarely, if ever, occurs. Patients who clear the virus spontaneously prior to the onset of cirrhosis do not develop liver complication as a result of HCV in later life.

Risks for disease progression

Patients who fail to clear the hepatitis C spontaneously or after treatment remain at risk for progression to liver failure and HCC (Figure 33.2). In one large hemophilia study which followed the cohorts from Sheffield, Utrecht, and Royal Free Hospital in London, 15% of patients developed liver failure or hepatocellular carcinoma after 30 years. The risk was highest among HIV-positive patients who did not receive antiretroviral therapy, in whom the risk of liver failure was

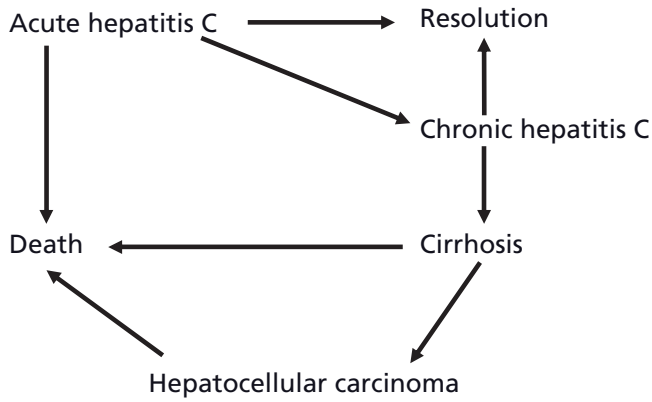

Figure 33.1 The natural history of hepatitis C.

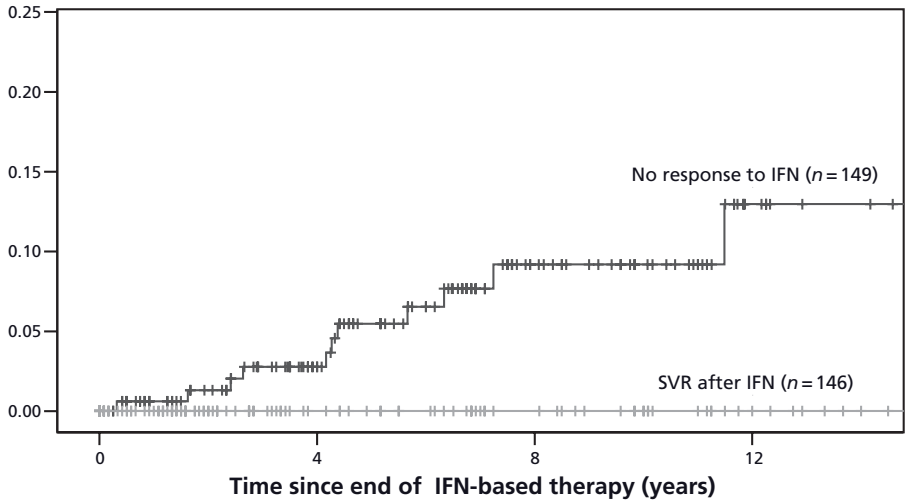

Figure 33.2 Risk of end-stage liver disease in hemophilia. Patients who fail to respond to interferon and ribavirin continue to have a high risk of liver failure. Reproduced from [7] with permission.

40% at 30 years. Risk factors for liver disease progression in hemophilia include higher age at first exposure, longer length of time since infection, infection with genotype 1, high levels of alcohol intake, and HIV coinfection [10].

Extrahepatic manifestations of chronic HCV

Several extrahepatic manifestations of HCV have been reported including mixed cryoglobulinemia, non-Hodgkin lymphoma, porphyria cutanea tarda, lichen planus, hypothyroidism, and Sjögren syndrome [11]. The presence of extrahepatic manifestations is an indication for HCV treatment even in the absence of marked liver disease.

Treatment

Available treatments

The first patients with hemophilia were treated with interferon in 1987, but response rates were low. The addition of ribavirin to interferon in 1995 and the subsequent pegylation of the interferon molecule have led to significant improvements in response rates [12]. The current standard of care for treatment of hepatitis C is the combination of pegylated interferon and ribavirin for 12 months in patients with genotypes 1, 4, 5, and 6, and 6 months in individuals with genotypes 2 and 3. Table 33.3 shows the definitions used in the treatment of hepatitis C and Table 33.4 gives the recommended treatments for chronic HCV.

Responses in nonhemophiliacs

In nonhemophiliacs the sustained virological response rate for genotype 1 is 40–50%, whereas for genotypes 2 and 3 it is 70–80%. Relapse rates remain relatively high (30% in patients with cirrhosis as a result of genotype 3 infection). Pegylated interferon and ribavirin is available from two manufacturers (Roche and Schering-Plough) but there are no major differences in the response rates between these two interferons.

Table 33.4 Recommended treatment schedules for chronic hepatitis C (HCV).

Genotype 1
Treat with pegylated interferon and ribavirin for 48 weeks
If HCV RNA level has not fallen by 2 logs by 12 weeks or not negative at 24 weeks, SVR is unlikely and treatment can be stopped
Genotypes 2 or 3
Treat with pegylated interferon and ribavirin for 24 weeks
If HCV RNA is negative at 4 weeks, treatment can be reduced to 12–16 weeks
HIV/HCV coinfected patients
Treat for 48 weeks irrespective of genotype

Early virological response

The kinetics of response to treatment in the first 12 weeks of therapy are very useful in predicting sustained virological response. Rapid virological response (RVR) is defined as a negative HCV RNA by PCR 28 days after commencing treatment. Complete early virological response (cEVR) is defined as a negative HCV RNA by PCR at 12 weeks. Partial EVR (pEVR) is defined as a ≥2 log decrease in viral load at week 12. RVR presages a sustained virological response. Individuals with pEVR have a 75% chance of achieving SVR. Similarly, patients who do not achieve EVR have a >95% chance they will not achieve SVR.

Responses in hemophiliacs to treatment in treatment-naive patients

Responses to pegylated interferon and ribavirin in hemophiliacs are very similar to those in nonhemophiliacs. Posthouwer and colleagues reported a 59% SVR response rate in hemophiliacs who had never been exposed to anti-HCV treatment previously. Although the response rate was lower in HIV-positive patients, it was still 32%, making this a very worthwhile subgroup to treat. Patients who clear the HCV with treatment have a dramatically reduced risk of progression of their liver disease. None of the 146 hemophiliacs in the Posthouwer study who obtained an SVR progressed up to 15 years of follow-up [7].

Responses to treatment in nonresponders and relapsers

Many patients with hemophilia were previously treated with either interferon monotherapy or with the nonpegylated form of interferon. It is well recognized that the responses to these treatments were inferior to the current standard of pegylated interferon and ribavirin. All individuals who failed to eliminate the virus with the older forms of treatments should be offered re-treatment with pegylated interferon and ribavirin. This has been shown to result in SVR in 39% of re-treated patients [7]. Patients who previously relapsed are more likely to achieve an SVR than prior nonresponders. There is no benefit in re-treating patients who fail a course of pegylated interferon and ribavirin.

Adverse events of treatment

Adverse events during treatment are common and lead to premature discontinuation of treatment in 10–20% of patients. Table 33.5 lists the adverse effects occurring in >1% of treated patients. Influenza-like symptoms are seen most frequently and their tolerance can be increased by the use of paracetamol/acetaminophen and taking the interferon dose at times of reduced activity such as the weekends. Depression is frequently troublesome, especially in patients with a history of the disease.

Table 33.5 Adverse events of treatment with pegylated interferon and ribavirin. Only events occurring in >1% of patients are shown

Influenza-like symptoms (fever, malaise, myalgia, nausea, athralgia, anorexia)
Site of injection symptoms (pain, erythema)
Neuropsychiatric (depression, anxiety, memory loss, insomnia, mood swings)
Dermatologic (reversible hair loss, rash, photosensitivity)
Hematologic (anemia, neutropenia, thrombocytopenia)
Autoimmune (induction of autoantibodies, especially thyroid)

Antidepressant medication is very useful both as treatment and prophylaxis. Patients with a severe neuropsychiatric history should be assessed by a psychiatrist prior to treatment. Cytopenias are usually mild and can be managed by dose adjustment of ribavirin in case of anemia and interferon in case of neutropenia or thrombocytopenia. In severe cytopenias, appropriate growth factors such as erythropoietin, granulocyte colony-stimulating factor and eltrombopag can be used. A rare but serious complication of particular interest to hemophilia is the development of anti-FVIII antibodies which can develop for the first time in patients with congenital hemophilia whilst having treatment or as autoantibodies (acquired hemophilia) in patients without a history of a bleeding disorder.

Treatment of patients with normal transaminases (ALT/AST)

Approximately a third of patients with chronic HCV have persistently normal aminotransferases; these individuals have less inflammation and fibrosis on liver biopsy and exhibit a more benign natural history. A total of 15–20% of these patients, however, have advanced fibrosis or cirrhosis and can develop liver failure and HCC [13]. Patients with normal ALT and AST have a similar response to interferon and ribavirin as other patients [14]. The decision to treat these patients is often difficult, especially in hemophiliacs where a liver biopsy is not available. This is actually one of the few situations where a liver biopsy does help in hemophilia because the identification of advanced disease would lead to a recommendation to treat. We would recommend that in the absence of treating all patients with normal serum aminotransferases, the following subgroups should be given priority: patients with advanced disease on biopsy or on noninvasive imaging/testing, patients with genotype 2/3 because of the high response rate, patients who wish to become pregnant, patients with symptomatic HCV such as those with severe tiredness or extrahepatic manifestations, and highly motivated individuals who wish to be hepatitis free.

Treatment of patients with cirrhosis

Patients with advanced liver disease may decompensate during treatment with interferon and ribavirin. It is, however, useful to treat patients with cirrhosis and monitor them carefully. Although only around 30% of the patients achieve SVR, those who do so have an 80% less risk of liver failure or HCC development [15]. Despite this reduced risk for the development of complications, individuals with documented cirrhosis should continue to have surveillance for HCC.

Treatment of HIV-coinfected patients

HCV progresses more rapidly in HIV-coinfected patients and these patients should be considered for treatment. This is best done in patients with a CD4 count of $>200 \times 10^6$/L.

Because of the risk of severe hemolysis, patients who are taking zidovudine (AZT), didanosine (ddI), or stavudine (d4T) should have their antiretroviral therapy modified so as to avoid these drugs during ribavirin therapy. The risk of adverse events in general is higher in coinfected patients. Coinfected patients should be treated for a total of 48 weeks irrespective of genotype.

Future treatments

The current treatment standard of pegylated interferon and ribavirin fails to cure up to 60% of treated patients and is associated with significant side-effects.

Current therapy for chronic hepatitis C is effective in approximately 50% of patients. There is an urgent need to develop improved therapies for these patients and to improve the outcomes in prior nonresponders to pegylated interferon and ribavirin. New antiviral therapies are being developed which include antagonists of specific targets of the HCV. Direct antivirals are very likely to form part of new treatment paradigms in the not too distant future.

Many new compounds are in development. These include nucleoside protease inhibitors, polymerase inhibitors, and non-nucleoside polymerase inhibitors. NS5a inhibitors have also been tested in phase I trials. New immunomodulatory therapies are currently being assessed. Several of these compounds are in phase I trial. Two protease inhibitors, i.e., the peptidomimetic inhibitors boceprevir and telaprevir, are in phase III trial. Results from phase II studies indicate that improved sustained virological response rates can be achieved in genotype 1-naive patients compared with the standard of care with these compounds. It is not clear how treatment with these agents can be optimized. It is also not clear whether a lead in strategy is important. Stopping rules have not been developed and predictors of response are not yet clear. Resistance is likely to remain an important issue, making testing of the agents in phase I somewhat problematic. Also, a new array of side-effects is being observed with these agents which will require appropriate management by hepatologists and infectious disease specialists. However, initial data suggest that therapy for HCV genotype 1-naive patients may be shortened, at least for a subgroup of patients with evidence of a rapid virus response, i.e., those who become HCV RNA undetectable by 4 weeks of therapy.

The effect of these new agents on different genotypes is incompletely studied and there may be emerging differences in efficacy against genotype 1a versus genotype 1b. Cross-resistance may prove an important barrier to effective treatment. In part, pegylated interferon may abrogate the effect of resistance.

These agents cannot be given as monotherapy. Initial data indicate that ribavirin will form an essential constituent of treatment for the time being. Ribavirin analogues that are less likely to cause anemia are being assessed. Several agents have been discarded for toxicity. In contrast to protease inhibitors, polymerase inhibitors theoretically have a higher genetic barrier to resistance. Only modest suppression has been observed with several of these agents so far, however. A combination of agents, for example R7128, with interferon and ribavirin has led to five log10 suppression of HCV RNA at 4 weeks.

Although the timeframe for achieving the ideal of dispensing with interferon is long, there may be an opportunity in the future for the use of a combination of oral agents in the treatment of hepatitis C. Oral treatments and agents with lower toxicity will improve treatment in the community and have a major societal impact on the existing burden of disease [16,17].

Hepatocellular carcinoma

Hepatocellular carcinoma is a malignant tumor arising in hepatocytes and is a complication occurring mainly in cirrhotic livers. HCC is increasingly being seen in hemophiliacs. Regular monitoring for HCC should be performed in hemophiliacs with cirrhosis, but in the absence of liver biopsies it may be difficult to identify cirrhotic individuals. Noninvasive testing with the fibrotest or fibroscan may help refine the population for monitoring but whether they identify all the patients at risk in hemophilia has not yet been confirmed. The two main means of monitoring for HCC are ultrasound scanning of the liver and measurement of serum α-fetoprotein. Ultrasound scanning is more sensitive at tumor detection. α-Fetoprotein may be elevated because of cirrhosis in the absence of HCC. The most cost-effective surveillance strategy is ultrasound imaging and α-fetoprotein measurement every 6 months [18].

HCC, when isolated and not bridging the liver capsule, can be treated with resection or liver transplantation, which can be curative. Palliative treatments include ethanol injection into the tumor, radiofrequency ablation and transarterial embolization.

Liver transplantation

Liver transplantation offers a cure to hemophiliacs with liver failure or isolated HCC, and these patients should be referred for the procedure in the same way as other patients with these complications. More than 50 transplants have been carried out in hemophiliacs worldwide. Because the liver is the site of production of factors VIII and IX, liver transplantation cures the hemophilia phenotypically, making it a highly cost-effective procedure, especially in patients with severe hemophilia [19,20]. Liver transplantation is not contraindicated in HIV-positive individuals provided the HIV is fully suppressed with highly active antiretroviral therapy (HAART).

Post liver transplantation, the new liver is invariably re-infected with HCV if the recipient was HCV RNA-positive going into transplantation, as is usually the case. Furthermore, the natural history of HCV post transplant is altered and the median time to cirrhosis is abbreviated to only 10–12 years, with survival after decompensation of only 40% at 1 year [21]. Patients with established HCV infection post liver transplantation should be treated with interferon and ribavirin.

References

1 Committee for Proprietary Medicinal Products (CPMP). Note for guidance on plasma derived products. Document number CPMP/BWP/269/95 rev. 3, 2001.

2 Richardson LC, Evatt BL. Risk of hepatitis A virus infection in persons with hemophilia receiving plasma-derived products. *Transfus Med Rev* 2000; **14**: 64–73.

3 Rumi MG, Colombo M, Gringeri A, *et al*. High prevalence of antibody to hepatitis C virus in multitransfused hemophiliacs with normal transaminase levels. *Ann Intern Med* 1990; **112**: 379–80.

4 Preston FE, Jarvis LM, Makris M, Philp L, Underwood JC, Ludlam CA, Simmonds P. Heterogeneity of hepatitis C virus genotypes in hemophilia: relationship with chronic liver disease. *Blood* 1995; **85**: 1259–62.

5 Alberti A, Morsica G, Chemello L, *et al*. Hepatitis C viraemia and liver disease in symptom free individuals with anti-HCV. *Lancet* 1992; **340**: 697–8.

6 Maor Y, Bashari D, Kenet G, Lubetsky A, Luboshitz J, Shapiro JM. Non-invasive biomarkers of liver fibrosis in haemophilia patients with hepatitis C: can you avoid liver biopsy? *Haemophilia* 2006; **12**: 372–9.

7 Posthouwer D, Yee TT, Makris M, *et al*. Antiviral therapy for chronic hepatitis C in patients with inherited bleeding disorders: an international, multicenter cohort study. *J Thromb Haemost* 2007b; **5**: 1624–9.

8 Piccininio F, Sagnelli E, Pasquale G, *et al*. Complications following percutaneous liver biopsy. *J Hepatol* 1986; **2**: 165–73.

9 Makris M, Preston FE. Liver biopsy in haemophilia. *Br J Haematol* 1997; **97**: 689–90.

10 Posthouwer D, Makris M, Yee TT, *et al*. Progression to end-stage liver disease in patients with inherited bleeding disorders and hepatitis C: an international, multicenter cohort study. *Blood* 2007a; **109**: 3667–71.

11 Galossi A, Guarisco R, Bellis L, Puoti C. Extrahepatic manifestations of chronic HCV infection. *J Gastrointestin Liver Dis* 2007; **16**: 65–73.

12 Posthouwer D, Mauser-Bunschoten EP, Fischer K, Makris M. Treatment of chronic hepatitis C in patients with haemophilia: a review of the literature. *Haemophilia* 2006; **12**: 473–8.

13 Puoti C, Bellis L, Galossi A, *et al*. Antiviral treatment of HCV carriers with persistently normal ALT levels. *Mini Rev Med Chem* 2008; **8**: 150–2.
14 Jacobson IM, Ahmed F, Russo MW, *et al*. Interferon alpha-2b and ribavirin for patients with chronic hepatitis C and normal ALT. *Am J Gastroenterol* 2004; **99**: 1700–5.
15 Veldt BJ, Heathcote J, Wedemeyer H, *et al*. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann Intern Med* 2007; **147**: 677–84.
16 Deutsch M, Hadziyannis SJ. Old and emerging therapies in chronic hepatitis C: an update. *J Viral Hepat* 2008; **15**: 2–11.
17 Cross TJS, Antoniades CG, Harrison PM. Current and future management of chronic hepatitis C infection. *Postgrad Med J* 2008; **84**: 172–6.
18 Thompson Coon J, Rogers G, Hewson P, *et al*. Surveillance of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis. *Health Technol Assess* 2007; **11**: 1–206.
19 Gordon FH, Mistry PK, Sabin CA, Lee CA. Outcome of orthotopic liver transplantation in patients with haemophilia. *Gut* 1998; **42**: 744–9.
20 Wilde J, Teixeira P, Bramhall SR, Gunson B, Mutimer D, Mirza DF. Liver transplantation in haemophilia. *Br J Haematol* 2002; **117**: 952–6.
21 Picciotto A. Antihepatitis C virus therapy in liver transplanted patients. *Ther Clin Risk Manag* 2006; **2**: 39–44.

34 Transfusion-transmitted disease: emerging infections

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Introduction

This chapter will focus on those agents which could be considered to be of potential relevance for the pathogen safety of plasma-derived hemophilia treatment products. As effective aseptic practices have long been embedded into the manufacturing processes of plasma derivatives, bacterial, fungal, and parasitic agents such as *Plasmodium*, *Babesia*, and *Borrelia*, that have been or potentially could be transfusion-transmitted [1], will thus not be included.

Human immunodeficiency virus (HIV) and the hepatitis B and C viruses, while so far the clinically most important causes for infectious adverse events associated with the treatment of hemophilia by plasma derivatives, are also excluded, as, given our current level of understanding, they can hardly be considered emerging agents any more, and, in addition, they are covered elsewhere in this book.

The infectious agents that remain of concern are emerging viruses and prions.

During the last decade some new viruses have been identified in human blood, often by novel molecular methods. Some of these viruses are in search of an associated disease entity, and pathogenicity for humans remains unsubstantiated.

During the same period, a number of other viruses have caused epidemics, often facilitated by changes in the ecologic environment to favor zoonotic risk factors, or the introduction of previously known agents into naive populations by global commerce or travel.

Equally, bovine spongiform encephalopathy (BSE) and variant Creutzfeldt–Jacob disease (vCJD) outbreaks have occurred, particularly in the UK.

Lipid-enveloped viruses

Some of the lipid-enveloped viruses found in blood have not been pathogenic for humans. For example, the GBV-C/hepatitis G virus (HGV) was shown to be transfusion transmissible [2], yet pathogenicity for humans has remained elusive [3]. On the contrary, GBV-C/HGV infection was even shown to result in slower disease progression of HIV coinfection [4].

The emergence of the severe acute respiratory syndrome (SARS) coronavirus in southern China represented a different level of challenge for human health, with 8098 humans clinically infected, of whom 774 succumbed to their infection, in an epidemic that spread to 29 countries [5] within a matter of weeks, before circulation of the virus could be halted by significant public health efforts. The demonstration of the virus in human blood, although at low levels and only during clinical stages of the disease [6], raised particular concern for the safety of hemophilia treatment. However, transfusion transmission of the SARS virus was never reported. In addition, virus reduction studies that used another coronavirus (Baxter GPS, unpublished), a physicochemically closely related so-called "model virus" [7], provided evidence that the SARS coronavirus should be inactivated during the manufacturing processes of plasma derivatives, which was later confirmed by limited investigations using the SARS coronavirus itself [8,9].

Other emerging lipid-enveloped viruses have actually caused blood transfusion transmissions, raising concerns for the safety of plasma derivatives.

After the 1999 introduction of West Nile virus (WNV) into the USA, the virus has emerged to endemicity in almost the entire nation, with an estimated past infection cumulatively in approximately 1% of the entire population [10]. As a consequence of the first few blood transfusion transmissions, nationwide screening of the blood supply by nucleic acid amplification technology (NAT) has been implemented. However, despite using the most modern technology available, the reduction of virus transmissions by blood transfusion was limited to a suboptimal 93% [11].

The plasma derivatives for major contribution of virus reduction, i.e., virus inactivation and removal that occurs during the manufacturing process, has been verified as effective against WNV [12].

This virus reduction capacity for flaviviruses can also provide reassurance: although there has been a significant level of emergence of these viruses, with a commensurate level of blood transfusion transmissions of, for example, dengue virus [13], there is no implication for the safety of plasma derivatives.

Another group of lipid-enveloped viruses that have been high on the global alert list are the avian influenza viruses, and specifically the highly pathogenic H5N1 virus, which has

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caused 393 reported human infections as of January 2009 (available from http://www.who.int/csr/disease/avian_influenza/country/en/), of which 248 (63%) have had a fatal outcome. Historic evidence for similar, and actually less pathogenic, influenza viruses supported the occurrence of preclinical viremia for influenza viruses [14], which has also been confirmed for the H5N1 virus itself [15]. In an attempt to achieve a more definitive understanding of the virus behavior during the manufacturing processes of plasma derivatives, the H5N1 virus was studied in *verification* studies, and was shown to be effectively inactivated by the most commonly used virus inactivation techniques, such as vapor heating, solvent detergent- and low pH-treatment, as well as pasteurization [16].

Infectious agents within the group of lipid-enveloped viruses are now understood as an insignificant challenge to the safety margins of modern plasma derivatives, particularly given the effectiveness of current virus reduction procedures.

Nonlipid-enveloped viruses

As the effectiveness of many virus inactivation and removal technologies is more limited against nonlipid-enveloped viruses, owing to their typically somewhat higher physicochemical resistance and often smaller size, respectively, emerging viruses of this class can potentially be a more significant remaining concern. Fortunately, though, they have so far been associated with more moderate disease in humans, as compared with many of their lipid-enveloped counterparts. In fact, attempts at demonstrating an association with any clinical disease in humans has been difficult or unsuccessful for some of these viruses.

For example, the initial identification of Torque teno virus (TTV) [17] and the related SEN-V (reported on July 20, 1999, in the *New York Times*) as blood transfusion-transmissible viruses understandably sparked public health concerns. Specifically for hemophilia, it was later reported that at least TTV could also occur as a contaminant of plasma-derivatives with less stringent purification processes [18]. Early reports even suggested that first-generation recombinant factor VIII (FVIII) products, through the albumin used as a stabilizer [19], could contain the virus, but this has been refuted by additional experiments [20]. However, no pathogenicity for humans has been substantiated to date, and so these viruses might be considered a commensal part of the microbiologic flora much rather than a pathogen.

The detection of the B19 parvovirus (B19V) in human plasma for fractionation was another concern for the treatment of hemophilia, particularly as the virus was suggested to reach uniquely high titers of up to 10E14 particles per milliliter [21], a number that would correspond to approximately 0.1% of the blood volume. The virus was assumed to be mostly resistant to inactivation, based on studies conducted with animal parvoviruses as models.

Where virus reduction did not seem a promising approach, the implementation of nucleic acid testing (NAT) to limit the load of plasma pools for manufacturing, initially as voluntary industry standard developed by the Plasma Proteins Therapeutics Association (PPTA), was a first step to enhance safety margins. In retrospect, these testing interventions have been one of the most successful ever, as evidenced particularly by a comparison with the more recent introduction of WNV NAT. Using WNV NAT, the risk of WNV transfusion transmission was reduced to only 7% of the initial value, i.e., by approximately 10-fold or 1 $\log_{10}$, whereas B19V NAT has reduced the average plasma pool load from initially 10 000 000 to now only 100 copies per mL, i.e., a reduction of 100 000-fold or 5 $\log_{10}$.

The subsequent recognition of the closely related erythroviruses V9 [22] and A6 [23], now reclassified as B19V genotypes 2 and 3, has necessitated reliable detection of all genotypic variants and, therefore, been a challenge for the diagnostic tests used to reduce the virus load of plasma for fractionation.

The use of new infectivity assays based on B19V-susceptible cell lines coupled with a molecular biology readout has demonstrated the significantly greater sensitivity of the different B19 viruses to liquid heating [24], low pH [24,25], and vapor heating [26], as compared with earlier used animal parvovirus models.

As already suggested by the clinical experience with plasma for transfusion which occasionally transmitted B19V when it contained virus above a certain threshold concentration [27], antibody-mediated B19V neutralization provides additional safety margins [28].

Whether the more distantly related PARV4 and bocaviruses will turn out to be a challenge for the safety of hemophilia treatment products is uncertain. These viruses are found in plasma for fractionation and even historic plasma derivatives [29], but clinically they do not seem to represent a major issue for human health.

Recent reports have suggested the transfusion transmission of hepatitis E virus (HEV) in Japan [30], i.e., a country where food-borne zoonotic HEV infections had also been recognized. A significant prevalence of the virus in the pig meat supply or even a few human infections have also reported from other countries, including the USA [31] and Germany [32]. As yet, a virus infectivity assay that would enable assessment of HEV reduction during the manufacturing of hemophilia treatment products is not widely available. The limited investigations that have been performed have shown the virus to be sensitive to inactivation [33]. In support of this finding, hemophilia patients treated with historic noninactivated products carry HEV antibodies more often than those only treated with inactivated products [34]. Thus, current limited experimental evidence would suggest that HEV is probably not a significant concern for the safety of plasma derivatives.

Most importantly, though, nonlipid-enveloped viruses are physiochemically somewhat resistant and they have shown

continued adaption. Therefore, vigilance needs to be supported by continued research into the physicochemical nature of these agents and their corresponding behavior during plasma fractionation processes, so that informed decisions can be taken by industry and regulators in a timely fashion.

Prions

When, in the mid-1980s, initial cases of bovine spongiform encephalopathy (BSE, also known as "mad cow disease") were recognized, at first in the UK, the public and political level of concern with respect to human health was rather low. Approximately a decade later, however, the emergence of variant Creutzfeldt–Jacob disease (vCJD) as a new disease entity of humans was identified, and more quickly presumed to be related to the (dietary) exposure of humans to the BSE agent [35]. These new findings triggered a great level of public concern, also around the use of bovine auxiliaries and excipients during the manufacture of medicinal products, including certain plasma derivatives as well as earlier-generation recombinant alternatives for the treatment of hemophilia. When finally blood transfusion transmissions of the vCJD agent became a possibility [36], the safety of plasma derivatives was equally questioned, with little appreciation for the intrinsic differences between directly transfused blood components versus manufactured plasma products that undergo purification processes designed to remove impurities, and thus intrinsically also pathogens that could potentially be present in the starting material.

Quickly, a number of additional procedures were put in place to safeguard both, bovine excipients as well as plasma derivatives, against the now realized concerns. The approach taken was comparably simple for bovine excipients, in that—most importantly—sourcing of the bovine raw materials was limited to "BSE-free" countries. However, a solution for plasma derivatives was not equally trivial, as neither a strongly associated risk factor that might have been used for the deferral of donors at risk, nor a test that could reliably predict the development of vCJD already at preclinical stages was, and still to date is, available. The entire weight was thus on the third and typically most effective leg of the safety tripod [37], beyond donor selection and donation testing, i.e., the reduction capacity of the manufacturing process.

A significant effort was thus made, primarily at laboratories of the plasma products industry, to investigate the behavior of prion agents during the manufacturing processes of plasma derivatives, as well as bovine excipients and auxiliaries. In these studies, the removal of the prion protein during manufacturing steps estimated to be particularly effective based on scientific evidence was mostly detected by Western blotting *in vitro*, or when needed for confirmatory purposes using the significantly more laborious and animal-consuming *in vivo* infectivity assays in small rodents, a procedure now also embedded in regulatory guidance on such investigations [38].

From the converging evidence derived from these studies it is now understood that prion proteins behave not unlike any other proteinaceous impurity during the relevant purification processes of, for example, plasma proteins, i.e., they are removed during manufacture of the final product by several orders of magnitude [39–41]. Combined with the already low concentrations of prion infectivity which might be present in the starting material such as plasma, for example, the information now available would support significant margins of safety for hemophilia treatment products.

Outlook

The inception of hemophilia treatment, initially with plasma-derived clotting factor concentrates, has dramatically changed the life expectancy and quality of those affected. It has also brought about the risk of infectious disease transmission. With the implementation of effective virus inactivation/removal processes, supported by virus marker testing and donor selection, the safety margins of these products were significantly enhanced, and current plasma derivatives enjoy substantial safety margins with respect to known infectious agents. Given the particularly robust reduction capacity for lipid-enveloped viruses, one might predict that any future emerging viruses within this class will not represent a significant challenge, whereas for nonlipid-enveloped viruses a higher level of vigilance and more frequent experimental work to verify our assumptions will remain prudent.

The advent of an entirely unappreciated class of pathogens, i.e., prion agents, has emphasized that any use of an animal- or human-derived component anywhere in the manufacturing process may lead to concerns around the potential presence and transmissibility of a pathogenic agent contained in these, even when using recombinant production techniques.

Fortunately, further technologic advances increasingly allow the production of recombinant clotting factors from production platforms entirely free of any exposure to human or animal proteins, thus finally eliminating any blood-borne infectious disease concerns as historically associated also with these pharmaceuticals.

References

1 Alter HJ, Stramer SL, Dodd RY. Emerging infectious diseases that threaten the blood supply. *Semin Hematol* 2007; **44**: 32–41.
2 Linnen J, Wages J, Jr., Zhang-Keck ZY, *et al*. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* 1996; **271**: 505–8.
3 Alter HJ. G-pers creepers, where'd you get those papers? A reassessment of the literature on the hepatitis G virus. *Transfusion* 1997; **37**: 569–72.
4 Lefrere JJ, Roudot-Thoraval F, Morand-Joubert L, *et al*. Carriage of GB virus C/hepatitis G virus RNA is associated with a slower immunologic, virologic, and clinical progression of human immu-

nodeficiency virus disease in coinfected persons. *J Infect Dis* 1999; **179**: 783–9.
5 MMWR. Revised U.S. Surveillance Case Definition for Severe Acute Respiratory Syndrome (SARS) and Update on SARS Cases—United States and Worldwide, December 2003. *MMWR* 2003; **52**: 1202–6.
6 Drosten C, Gunther S, Preiser W, van der WS, Brodt HR, Becker S, *et al*. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 2003; **348**: 1967–76.
7 EMEA. *CPMP Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses* (CPMP/BWP/268/95/rev). CPMP/BWP/268/95.
8 Darnell ME, Taylor DR. Evaluation of inactivation methods for severe acute respiratory syndrome coronavirus in noncellular blood products. *Transfusion* 2006; **46**: 1770–7.
9 Yunoki M, Urayama T, Yamamoto I, Abe S, Ikuta K. Heat sensitivity of a SARS-associated coronavirus introduced into plasma products. *Vox Sang* 2004; **87**: 302–3.
10 Planitzer CB, Modrof J, Yu MYW, Kreil TR. West Nile virus infection in plasma of blood and plasma donors, United States. *Energ Infect Dis* 2009; **15**: 1668–70.
11 Petersen LR, Epstein JS. Problem solved? West Nile virus and transfusion safety. *N Engl J Med* 2005; **353**: 516–17.
12 Kreil TR, Berting A, Kistner O, Kindermann J. West Nile virus and the safety of plasma derivatives: verification of high safety margins, and the validity of predictions based on model virus data. *Transfusion* 2003; **43**: 1023–8.
13 Tambyah PA, Koay ES, Poon ML, Lin RV, Ong BK. Dengue hemorrhagic fever transmitted by blood transfusion. *N Engl J Med* 2008; **359**: 1526–7.
14 Naficy K. Human influenza infection with proved viremia. Report of a case. *N Engl J Med* 1963; **269**: 964–6.
15 Chutinimitkul S, Bhattarakosol P, Srisuratanon S, *et al*. H5N1 influenza A virus and infected human plasma. *Emerg Infect Dis* 2006; **12**: 1041–3.
16 Kreil TR, Unger U, Orth SM, *et al*. H5N1 influenza virus and the safety of plasma products. *Transfusion* 2007; **47**: 452–9.
17 Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem Biophys Res Commun* 1997; **241**: 92–7.
18 Simmonds P, Davidson F, Lycett C, *et al*. Detection of a novel DNA virus (TTV) in blood donors and blood products. *Lancet* 1998; **352**: 191–5.
19 Azzi A, De SR, Morfini M, *et al*. TT virus contaminates first-generation recombinant factor VIII concentrates. *Blood* 2001; **98**: 2571–3.
20 Kreil TR, Zimmermann K, Pable S, Schwarz HP, Dorner F. TT virus does not contaminate first-generation recombinant factor VIII concentrate. *Blood* 2002; **100**: 2271–2.
21 Weimer T, Streichert S, Watson C, Groner A. High-titer screening PCR: a successful strategy for reducing the parvovirus B19 load in plasma pools for fractionation. *Transfusion* 2001; **41**: 1500–4.
22 Nguyen QT, Sifer C, Schneider V, *et al*. Novel human erythrovirus associated with transient aplastic anemia. *J Clin Microbiol* 1999; **37**: 2483–7.
23 Nguyen QT, Wong S, Heegaard ED, Brown KE. Identification and characterization of a second novel human erythrovirus variant, A6. *Virology* 2002; **301**: 374–80.
24 Blumel J, Schmidt I, Willkommen H, Lower J. Inactivation of parvovirus B19 during pasteurization of human serum albumin. *Transfusion* 2002; **42**: 1011–18.
25 Boschetti N, Niederhauser I, Kempf C, Stuhler A, Lower J, Blumel J. Different susceptibility of B19 virus and mice minute virus to low pH treatment. *Transfusion* 2004; **44**: 1079–86.
26 Berting A, Modrof J, Unger U, *et al*. Inactivation of parvovirus B19 during STIM-4 vapor heat treatment of three coagulation factor concentrates. *Transfusion* 2008; **48**: 1220–6.
27 Davenport RJ, Geohas G, Cohen S, *et al*. Phase IV Study of Plas+SD: Hepatitis A (HAV) and Parvovirus B19 (B19) safety results. *Blood* 2000; 96(Suppl.): 451a.
28 Modrof J, Berting A, Tille B, *et al*. Neutralization of human parvovirus B19 by plasma and intravenous immunoglobulins. *Transfusion* 2007; **48**: 178–86.
29 Fryer JF, Hubbard AR, Baylis SA. Human parvovirus PARV4 in clotting factor VIII concentrates. *Vox Sang* 2007; **93**: 341–7.
30 Mitsui T, Tsukamoto Y, Yamazaki C, *et al*. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. *J Med Virol* 2004; **74**: 563–72.
31 Feagins AR, Opriessnig T, Guenette DK, Halbur PG, Meng XJ. Detection and characterization of infectious Hepatitis E virus from commercial pig livers sold in local grocery stores in the USA. *J Gen Virol* 2007; **88**: 912–17.
32 Wichmann O, Schimanski S, Koch J, *et al*. Phylogenetic and case–control study on hepatitis E Virus infection in Germany. *J Infect Dis* 2008; **198**: 1732–41.
33 Yunoki M, Yamamoto S, Tanaka H, e*t al*. Extent of hepatitis E virus elimination is affected by stabilizers present in plasma products and pore size of nanofilters. *Vox Sang* 2008; **95**: 94–100.
34 Toyoda H, Honda T, Hayashi K, e*t al*. Prevalence of hepatitis E virus IgG antibody in Japanese patients with hemophilia. *Intervirology* 2008; **51**: 21–5.
35 Will RG, Ironside JW, Zeidler M, e*t al*. A new variant of Creutzfeldt–Jakob disease in the UK. *Lancet* 1996; **347**: 921–5.
36 Llewelyn CA, Hewitt PE, Knight RS, e*t al*. Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. *Lancet* 2004; **363**: 417–21.
37 Farrugia A. The mantra of blood safety: time for a new tune? *Vox Sang* 2004; **86**: 1–7.
38 EMEA. G*uideline on the investigation of manufacturing processes for plasma-derived medicinal products with regard to VCJD risk* (CPMP/BWP/5136/03). CPMP/BWP/5136/03.
39 Cai K, Miller JL, Stenland CJ, e*t al*. Solvent-dependent precipitation of prion protein. *Biochim Biophys Acta* 2002; **1597**: 28–35.
40 Stenland CJ, Lee DC, Brown P, Petteway SR, Jr., Rubenstein R. Partitioning of human and sheep forms of the pathogenic prion protein during the purification of therapeutic proteins from human plasma. *Transfusion* 2002; **42**: 1497–500.
41 Vey M, Baron H, Weimer T, Groner A. Purity of spiking agent affects partitioning of prions in plasma protein purification. *Biologicals* 2002; **30**: 187–96.

35 Hemophilia gene therapy: an overview

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Since the recognition of gene therapy as a therapeutic paradigm, hemophilia has been a prime candidate disease for the successful application of this treatment approach [1]. The disease is a simple monogenic recessive trait and, as such, delivery of a normal coagulation factor in sufficient quantities to replace the missing or dysfunctional protein should rescue the mutant phenotype.

Rationale for gene transfer in hemophilia

In addition to being a very well-characterized recessive monogenic disease, hemophilia presents several other features that significantly enhance its appeal as a gene therapy candidate. Most importantly, extensive knowledge of the natural history of bleeding in patients with moderately severe hemophilia (clotting factor levels of 1–5%) indicates that spontaneous bleeding at these low levels of clotting factor is rare. Thus, attainment of sustained clotting factor levels of between 1% and 5% through gene transfer should minimize the risk for this disease complication.

The second pragmatic advantage for hemophilia gene transfer is the lack of requirement for tightly regulated factor levels. While, ideally, gene transfer should deliver levels within the normal physiologic range, there is good reason to believe that levels between 2% and 150% would benefit hemostasis and not promote an increased thrombogenic risk. In addition, as long as access to the circulation is ensured and appropriate post-translational processing has produced a fully functional protein, the cellular site of factor VIII (FVIII) expression is not limiting.

Finally, the preclinical evaluation of hemophilia gene transfer has been significantly facilitated through the availability of excellent small and large animal models of the disease [2]. Several mouse models of hemophilia A and B have been generated and there are colonies of hemophilia A and B dogs that have been used very successfully to evaluate the safety and efficacy of novel hemophilia therapeutics.

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Basic components of a gene transfer protocol

The therapeutic transgene

The aim of gene transfer programs is to deliver a therapeutic cargo to rescue a pathologic phenotype in the gene transfer recipient. In the hemophilias, this goal is accomplished by the delivery of sustained expression of clotting factor protein [FVIII or factor IX (FIX)] from a therapeutic transgene.

The transgene cassette

In hemophilia gene transfer protocols the transgene is almost always a contiguous cDNA sequence, although the inclusion of a 5′ intron has been helpful to boost expression levels in some instances. While the FIX cDNA (~1.3 kb) is readily contained in most vector systems, the FVIII cDNA (~8 kb) is not easily packaged in most of the viral vectors currently in use. For this reason, removal of the nonessential B domain of the FVIII cDNA sequence reducing the modified cDNA to ~4.5 kb has been carried out in most hemophilia A gene transfer studies. In addition to creating modified intronless cDNA constructs, some investigators have further "codon optimized" the cDNA sequence to enhance the potential for protein translation in targeted recipient cells.

Other components of the transgene cassette (Figure 35.1) that require consideration for optimal gene transfer outcomes include 5′ regulatory elements and 3′ noncoding sequence. In particular, the transgene promoter should be chosen with regard to the likely target cell type for transgene expression. While viral promoter elements provide strong transcriptional effects *in vitro*, in some instances these promoters are susceptible to transcriptional silencing [3]. Thus, many gene transfer protocols now utilize either native enhancer/promoter constructs or synthetic regulatory elements that ensure long-term expression following transgene delivery [4]. Nevertheless, despite these advances in promoter technology, the utility of optimal genomic regulatory elements is significantly constrained by the packaging capacity of most vector systems. At the 3′ end of the transgene cDNA a polyadenylation signal must be inserted and some 3′ noncoding sequences have the potential to stabilize the resulting mRNA.

Figure 35.1 Components of a therapeutic transgene cassette.

Table 35.1 Strategies for transgene delivery.

In Vivo	Direct administration into the recipient Intravenous, intramuscular, subcutaneous
Ex Vivo	Administration to cells outside of the body and subsequent implantation Hematopoietic stem cells, mesenchymal stem cells, endothelial progenitor cells

The transgene delivery protocol

Even with the most efficient transgene cassette, optimal *in vivo* expression requires an effective delivery system that ensures transport of the transgene to sufficient numbers of host target cells. While significant advances have been made in this area of gene therapy, the "perfect" delivery system does not exist, and a variety of delivery protocols are being used in hemophilia gene transfer studies.

In addition to a number of different delivery vehicles, gene transfer can be accomplished by either direct *in vivo* transgene administration such as through intravenous or intramuscular injection, or through delivery to host cells *ex vivo* in cell culture after their isolation from the host (Table 35.1). While *in vivo* delivery strategies are more pragmatic and more readily applicable to large patient populations, they are inevitably associated with an increased likelihood of early adverse effects resulting from the process of transgene delivery. In contrast, in *ex vivo* approaches, transgene delivery to the host cell occurs outside of the body and thus avoids potential innate immune responses to the delivery vehicle. *Ex vivo* delivery also enables direct targeting of the distinct cell population that has been harvested from the host, e.g., hematopoietic stem cells. Unless the transgene is administered by direct injection (e.g., intramuscular injection), *in vivo* transgene delivery must rely upon inherent (or engineered) tropic properties of the delivery vehicle to facilitate cell-type specific gene transfer. Finally, given the relative efficiencies of the two delivery approaches, the vector dose requirements for *in vivo* strategies will usually be much higher than for *ex vivo* delivery with the corresponding vehicle.

The transgene vehicle (Table 35.2)

Nonviral transgene delivery

In general, nonviral transgene delivery approaches are easier to develop than viral vector-mediated strategies. In most instances, this will involve the generation of transgene plasmid DNA that is subsequently administered by direct injection of the naked DNA or via a form of molecular conjugate. DNA conjugation protocols have been established with a wide range of compounds, all possessing protective properties to facilitate transgene delivery to cells in an intact form [5]. These compounds have ranged from cationic lipids, to various forms of nanoparticle structures and the linear polysaccharide chitosan. While the generation of these plasmid conjugates is variably straightforward to accomplish, the efficient targeting, cell entry, and nuclear transgene transport achieved by these conjugates continues to pose major challenges. In general, the transgene delivery process mediated through these strategies is still too inefficient to merit widespread clinical introduction.

Table 35.2 Transgene delivery vehicles and strategies.

Viral Vectors	Adenovirus Adeno-associated virus Lentivirus
Nonviral Delivery	Liposomes Nanoparticles Electroporation Direct injection

There have been exceptions to the limitations posed by inefficient delivery of plasmid DNA. Approaches using electroporation to facilitate cellular DNA entry have been developed and can even be applied locally *in vivo* [6]. In addition, hydrodynamic delivery strategies also continue to show promise [7]. In laboratory animals, this approach can be applied systemically, but recent evidence has suggested that hydrodynamic delivery to localized vascular beds can provide very effective transgene expression, although transient local tissue injury is inevitable with this technology [8].

In summary, while some form of nonviral vector-mediated gene transfer approach might ultimately be the optimal method to deliver therapeutic transgenes, there will need to be significant improvements in the efficiency of this process before widespread clinical application is realized.

Viral vector-mediated transgene delivery

Currently, the most effective way to deliver a transgene to recipient cells is through the utilization of one of a number of different viral vectors (Table 35.2 and 35.3). Viruses have evolved over millions of years to efficiently introduce their nucleic acid genomes into host cells and, to date, attempts to improve upon this process have been unsuccessful. Thus, over the past three decades, efforts have centered upon the generation of viral vectors that preserve the efficient delivery mechanisms inherent in native viruses but abolish the replication competency and pathogenic features of the virus [9]. These

Table 35.3 Optimal characteristics of a viral vector for use in gene therapy.

Optimal viral vector characteristics
High titer production achievable
Intravenous administration possible
Minimal (no) influence on innate immunity
Low prevalence of antibodies in the patient population
Efficient transduction of target cell population
Nonintegrating or targeted integration into host genome

approaches have been variably successful and small phase I/II clinical studies of hemophilia gene transfer have now been performed with three different types of viral vector: adenovirus, retrovirus and adeno-associated virus.

Adenoviral vectors

These double-stranded DNA viruses have been used for gene transfer for over 20 years. During this time, the viral genome has been progressively removed such that the current third generation of adenoviral vectors (helper-dependent or gutless vectors) only maintain the terminal repeat sequences and packaging sequence of the original viral genome. In these vectors, the structural viral proteins required for viral particle assembly are provided in "trans" through the cotransfection of packing cell lines with a helper adenovirus [10].

Adenoviral vectors have a number of characteristics that make them attractive candidates for gene transfer. They can fairly easily be grown to high titers and are extremely efficient at transducing many different cell types both *in vitro* and *in vivo*. Furthermore, in the latest generation of helper-dependent vectors, transgene inserts of up to 30 kb can be cloned into the vector backbone thus enabling large genomic regulatory sequences to be used for controlling transgene expression [11]. Another potential advantage of adenoviral-based vectors is that they do not integrate into the host cell genome, but remain for prolonged periods of time as stable extrachromosomal elements in nondividing cells. Thus, the risk of insertional mutagenesis is essentially eliminated with adenoviral vectors.

However, despite these various advantages, the major limitation of adenoviral vectors is the potent innate immune response that these vehicles elicit [12]. Even with the development of the most recent generation of helper-dependent adenoviral vectors, the adverse effects of the viral transduction process are still evident [13]. These effects are mediated by proinflammatory cytokine responses and result in a number of acute, transient pathologies in the hours and first few days after adenoviral vector administration. There is often evidence of transient cytotoxicity in the first 48 h after intravenous administration (most often hepatotoxicity), and during the first week after vector delivery, reversible thrombocytopenia is frequent [14]. With increasing vector doses, or in more susceptible hosts, these effects can be more profound and in its most extreme manifestation the innate immune response can be fatal as it results in a systemic inflammatory state [15]. While there are certain features of the transgene delivery protocol that are known to influence this outcome (i.e., vector dose and route of administration), there is still an element of unpredictability, which inevitably increases the risk associated with using systemically administered adenoviral vectors in the clinic. For this reason, the use of adenoviral vectors in hemophilia gene therapy is highly unlikely until further progress is made in mitigating the innate immune reactivity to this vector type.

Retroviral vectors

Retroviral vectors, derived from a large family of RNA viruses, have been used in gene transfer protocols for more than 20 years. The original gammaretroviral vectors were limited by their requirement for cell division to facilitate nuclear entry, but the more recent introduction of lentiviral-based vectors that can also transduce postmitotic cells has significantly broadened the potential for these transgene delivery vehicles [16]. As just one example, lentiviral vectors are excellent candidates for transduction of mitotically quiescent stem cells.

However, there are still limitations to the use of these vectors for gene transfer. While there has been some progress in the development of stable packaging cell lines, high-titer retroviral vector production is still not straightforward. Furthermore, the packaging constraints inherent in the use of retroviral vectors also limits transgene size to approximately 8 kb, a factor that adversely influences the types of regulatory sequences that can be used to mediate transgene expression, especially of large cDNAs such as FVIII.

Most importantly, and in contrast to adenoviral vectors, retroviruses integrate into the host cell genome as part of their natural life cycle. In the context of gene therapy, this ensures that the transgene will be stably carried by the host cell, even if a replicating cell population has been transduced. Until relatively recently, it had been thought that retroviral integration sites were randomly distributed throughout the genome. However, there is now good evidence to indicate that many integration sites coincide with regions of the genome that are transcriptionally active within the transduced cell (i.e., areas where the chromatin configuration is more open and accessible) [17,18]. There also appear to be differences in the integration site preferences between gammaretroviruses and lentiviruses with the former vectors more often integrating in *5′* upstream sequences close to transcription start sites and lentiviruses more often into the introns of transcriptionally active genes [19]. These observations help to explain the insertional mutagenesis outcomes that contributed to the leukemic

transformations documented in the recent severe combined immunodeficiency disease gene therapy studies. In these cases, delivery with a gammaretroviral vector and integration of the transgene adjacent to a gene involved in lymphocyte development (*LMO2*) appears to have resulted in the inappropriate expression of this gene product and the emergence of an uncontrolled lymphoproliferative state [20]. The ultimate expression of these integration events as leukemia very likely also involved critical contributions from the transgene product (a growth factor receptor) and additional acquired genetic anomalies. Nevertheless, these adverse clinical events have served as an important reminder of the potential problems that can result from the inopportune integration of these vectors. While targeting integration events to safe regions of the genome would be ideal, the practical realization of this strategy is still some way from application for gene therapy purposes.

Adeno-associated viral vectors

Adeno-associated viruses (AAVs) are small replication-defective, nonpathogenic parvoviruses that show a broad spectrum of cellular tropism in humans. Adeno-associated viruses possess a single-stranded DNA genome and require help from a replication-competent virus (most often adenovirus) to propagate. Recent investigation has revealed many new AAV serotypes that have the potential for expanding the therapeutic opportunities with AAV-based vectors.

After cell entry, AAV vectors persist within the nucleus as stable extrachromosomal concatemers with only very small amounts of the vector sequence (<5%) being integrated into the host genome [21]. Among the limiting factors associated with the use of AAV vectors is the small size of the AAV genome, which restricts the size of the transgene cassette to <5 kb, and the fact that large-scale production of AAV vectors requires special expertise.

To date, the most significant benefit realized by AAV vectors has been the minimal immunogenicity associated with these particles. In contrast to both adenoviral and, to a somewhat lesser extent, retroviral vectors, AAV vectors administered by a number of different routes appear to be associated with minimal perturbation of the innate immune system. No acute adverse effects have been reported following even high AAV vector doses and, with one exception, there has been no long-term pathology reported with the use of these vectors. The single exception relates to reports from one group of liver tumors developing in older mice that received an AAV vector to treat mucopolysaccharidosis type VII [22]. While subsequent integration site analysis of the tumor DNA from these mice showed a clustering of AAV integration events on chromosome 12, in a genomic region that encodes several imprinted genes and a number of miRNAs [23], there remains significant skepticism that these occurrences are reflective of a broader tumorigenic potential of AAV.

One additional problem that has influenced clinical outcomes with AAV vectors is the prevalence within humans of pre-existing AAV immunity [24]. Humoral immunity to AAV is present in the majority of humans but varies significantly with different AAV serotypes. While these anti-AAV antibodies will compromise the transduction efficiency of AAV-based vectors, the presence of pre-existing cellular immunity to the virus has proven equally challenging through the development of postadministration cytotoxic responses to AAV-transduced cells. While evidence for anti-AAV antibodies is relatively easy to ascertain, pre-existing T-cell immunity is extremely difficult to document.

The current development of plans for two AAV-based gene therapy trials in hemophilia B indicates that this vector system remains an excellent candidate for clinical application. There will be particular interest in the delayed cell-mediated immune responses following these vector administrations but, based on much preclinical and previous clinical investigation, these trials may result in long-term therapeutic expression of FIX.

Future challenges for gene therapy

While the discipline of gene therapy is now close to 30 years old, examples of clinical success remain very small in number. Successes with the treatment of infant patients with severe combined immunodeficiency disease has been tempered with the development of leukemia in some cases [25,26] while the successes seen in Leber's congenital amaurosis [27] and late infantile neuronal ceroid lipofuscinosis [28] are still in a very small number of patients and very early on for significant phenotypic improvements to be realized [29]. No gene transfer delivery vehicle is clearly superior at this time and there is still much to learn about optimizing transgene delivery and expression.

The major challenges for gene therapy that have still to be overcome are detailed in Table 35.4. For clinical studies, large-scale vector production will need to be improved and better forms of cellular targeting would be optimal. The use of all viral vector-based delivery protocols has the potential to activate innate immune reactivity and mitigation of this response

Table 35.4 Outstanding challenges to successful gene transfer.

Major outstanding gene transfer challenges
1. Host Immune responses
a) Innate immunity to the delivery vehicle
b) Adaptive immunity to the delivery vehicle
c) Adaptive immunity to the transgene protein
2. Efficient cell-targeted transduction
3. Targeted vector integration or long-term extrachromosomal transgene persistence
4. Persistent high-level transgene expression

will be an important component of any successful gene transfer trial [30]. In addition, adaptive immune responses to both the vector and the transgene product may also pose problems, especially in immunocompetent hosts with null phenotypes for inherited protein deficiencies (i.e., severe hemophilia) [31].

Despite the challenges that lie ahead, there is still a well-founded optimism that gene therapy will prove successful for providing long-term "cures" for protein deficiency states such as hemophilia. Where safe and effective therapies already exist, as they do in hemophilia, advances toward gene therapy must always weigh the balance between safety and potential therapeutic benefit. Further advances are awaited with great interest.

References

1 Murphy SL, High KA. Gene therapy for haemophilia. *Br J Haematol* 2008; **140**: 479–87.
2 Rawle FE, Lillicrap D. Preclinical animal models for hemophilia gene therapy: predictive value and limitations. *Semin Thromb Hemost* 2004; **30**: 205–13.
3 Ellis J. Silencing and variegation of gammaretrovirus and lentivirus vectors. *Hum Gene Ther* 2005; **16**: 1241–6.
4 Notley C, Killoran A, Cameron C, Wynd K, Hough C, Lillicrap D. The canine factor VIII 3′-untranslated region and a concatemeric hepatocyte nuclear factor 1 regulatory element enhance factor VIII transgene expression in vivo. *Hum Gene Ther* 2002; **13**: 1583–93.
5 Rettig GR, Rice KG. Non-viral gene delivery: from the needle to the nucleus. *Expert Opin Biol Ther* 2007; **7**: 799–808.
6 Miyazaki S, Miyazaki J. In vivo DNA electrotransfer into muscle. *Dev Growth Differ* 2008; **50**: 479–83.
7 Suda T, Liu D. Hydrodynamic gene delivery: its principles and applications. *Mol Ther* 2007; **15**: 2063–9.
8 Suda T, Gao X, Stolz DB, Liu D. Structural impact of hydrodynamic injection on mouse liver. *Gene Ther* 2007; **14**: 129–37.
9 Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. *Nat Med* 2001; **7**: 33–40.
10 Parks RJ, Chen L, Anton M, Sankar U, Rudnicki MA, Graham FL. A helper-dependent adenovirus vector system: removal of helper virus by Cre-mediated excision of the viral packaging signal. *Proc Natl Acad Sci USA* 1996; **93**: 13565–70.
11 Parks RJ, Graham FL. A helper-dependent system for adenovirus vector production helps define a lower limit for efficient DNA packaging. *J Virol* 1997; **71**: 3293–8.
12 Muruve DA. The innate immune response to adenovirus vectors. *Hum Gene Ther* 2004; **15**: 1157–66.
13 Muruve DA, Cotter MJ, Zaiss AK, *et al*. Helper-dependent adenovirus vectors elicit intact innate but attenuated adaptive host immune responses in vivo. *J Virol* 2004; **78**: 5966–72.
14 Othman M, Labelle A, Mazzetti I, Elbatarny HS, Lillicrap D. Adenovirus-induced thrombocytopenia: the role of von Willebrand factor and P-selectin in mediating accelerated platelet clearance. *Blood* 2007; **109**: 2832–9.
15 Raper SE, Chirmule N, Lee FS, *et al*. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol Genet Metab* 2003; **80**: 148–58.
16 Ailles LE, Naldini L. HIV-1-derived lentiviral vectors. *Curr Top Microbiol Immunol* 2002; **261**: 31–52.
17 Daniel R, Smith JA. Integration site selection by retroviral vectors: molecular mechanism and clinical consequences. *Hum Gene Ther* 2008; **19**: 557–68.
18 Bushman F, Lewinski M, Ciuffi A, *et al*. Genome-wide analysis of retroviral DNA integration. *Nat Rev Microbiol* 2005; **3**: 848–58.
19 Mitchell RS, Beitzel BF, Schroder AR, *et al*. Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences. *PLoS Biol* 2004; 2: E234.
20 Hacein-Bey-Abina S, von KC, Schmidt M, *et al*. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003; **302**: 415–19.
21 Nakai H, Wu X, Fuess S, *et al*. Large-scale molecular characterization of adeno-associated virus vector integration in mouse liver. *J Virol* 2005; **79**: 3606–14.
22 Donsante A, Vogler C, Muzyczka N, *et al*. Observed incidence of tumorigenesis in long-term rodent studies of rAAV vectors. *Gene Ther* 2001; **8**: 1343–6.
23 Donsante A, Miller DG, Li Y, Vogler C, Brunt EM, Russell DW, Sands MS. AAV vector integration sites in mouse hepatocellular carcinoma. *Science* 2007; 317: 477.
24 Zaiss AK, Muruve DA. Immunity to adeno-associated virus vectors in animals and humans: a continued challenge. *Gene Ther* 2008; **15**: 808–16.
25 Howe SJ, Mansour MR, Schwarzwaelder K, *et al*. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J Clin Invest* 2008; **118**: 3143–50.
26 Hacein-Bey-Abina S, Garrigue A, Wang GP, *et al*. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest* 2008; **118**: 3132–42.
27 Maguire AM, Simonelli F, Pierce EA, *et al*. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med* 2008; **358**: 2240–8.
28 Worgall S, Sondhi D, Hackett NR, *et al*. Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. *Hum Gene Ther* 2008; **19**: 463–74.
29 Wilson JM, Gansbacher B, Berns KI, *et al*. Good news on the clinical gene transfer front. *Hum Gene Ther* 2008; **19**: 429–30.
30 Brown BD, Lillicrap D. Dangerous liaisons: the role of "danger" signals in the immune response to gene therapy. *Blood* 2002; **100**: 1133–40.
31 Lillicrap D. Hemophilia treatment. Gene therapy, factor VIII antibodies and immune tolerance: hopes and concerns. *Haematologica* 2000; **85**: 108–11.

36 Gene therapy trials in hemophilia A and B

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The first clinical trial of gene therapy was begun in 1990 at the US National Institutes for Health (NIH), where a retroviral vector expressing the adenosine deaminase gene was introduced into the T lymphocytes of children with severe combined immunodeficiency disease (SCID). Although not a clearcut success because of low levels of gene transfer, the approach appeared safe, and many other trials followed. Beginning in 1998, five different trials of gene therapy for hemophilia were undertaken in a fairly short space of time (Table 36.1) [1]. As was the case for the initial trial for ADA-SCID, none resulted in long-term success, but all showed reasonable safety profiles and one showed at least short-term efficacy at the highest dose tested [2]. In retrospect, and with an additional decade of clinical experience in gene therapy, it is clear that only one of these trials—infusion of an adeno-associated virus (AAV) vector into the hepatic artery—had the advantage of robust preclinical data in a large animal model of the disease [3]; perhaps not surprisingly, this is the only one of the original group of studies still moving forward, an observation that underscores the importance of rigorous testing of proposed clinical strategies in large animal models of disease. Nonetheless, all of the studies were informative and provided data which form a starting point for a new generation of trials currently beginning. As is often the case for novel classes of therapeutics, several iterations may be required to attain the therapeutic goal—in this case, long-term expression of therapeutic levels of clotting factor from a single administration of vector. This chapter will review the previous trials, the preclinical studies on which they were based, and subsequent studies that have resulted in formulation of strategies that are more likely to achieve the desired therapeutic outcome.

To achieve the goal of gene therapy for genetic disease—long-term expression of the therapeutic transgene at levels high enough to improve the phenotype of the disease—two general strategies can be pursued. The first uses an integrating vector to transduce a stem cell, so that all daughter cells carry the donated gene. This ensures long-term expression but carries the risk of insertional mutagenesis related to the integration event(s). The second is to use a vector to transduce a long-lived postmitotic cell type. In this case, as long as the donated gene is stably maintained, there is no need for integration, since the cell itself is long-lived. This approach thus has the possibility to avoid risks related to insertional mutagenesis, but, because it generally requires *in vivo* gene delivery, carries another risk, that of immunotoxicity. Because long-lived postmitotic cells such as skeletal muscle or cells of the central nervous system (CNS) are not easily removed for manipulation in the laboratory, the vector must be administered *in vivo*, increasing the risk that an immune response to the vector will occur and perhaps promote or exacerbate an immune response to the transgene product as well.

In contrast to the situation at the start of the hemophilia gene therapy trials a decade ago, success using both of the strategies outlined above has now been demonstrated in both inherited and acquired disorders. The first clearcut success in gene therapy—the restoration of immune function in children with X-linked SCID [4]—used the strategy of transduction of stem cells, in this case $CD34^+$ hematopoietic stem cells, with an integrating retroviral vector. The strong *in vivo* selection for transduced cells helped to ensure success here, but also likely contributed to selection for clones with a growth advantage, resulting approximately 3 years after transduction in the occurrence of T-cell leukemia in some subjects [5,6]. Other trials that have used the strategy of *ex vivo* transduction of stem cells have included successful treatment of ADA-SCID in children [7,8], and treatment of severe junctional epidermolysis bullosa using transduction of epidermal skin cells [9]. [The failure to observe leukemias in children with ADA-SCID may be related to the less powerful selection pressure for transduced cells in this setting; in fact, in order to achieve engraftment a mild conditioning regimen was administered prior to infusion of transduced autologous cells, and replacement therapy with polyethylene glycol (PEG)-ADA was also withdrawn to provide some advantage for the transduced cells.] Success with the second strategy—*in vivo* transduction of long-lived postmitotic cell types—has now been achieved in at least two settings, the retinal degenerative disorder Leber's congenital amaurosis [10–12], and the neurodegenerative disorder Parkinson's disease [13]. The vector in both of these cases was recombinant AAV; injection of the vector into the

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Table 36.1 Gene transfer trials in hemophilia.

Sponsor	Hemophilia	Number of subjects	Administration	Reference
TKT	A	12	*Ex vivo*: electroporation of autologous fibroblases, re-implantation onto peritoneum	Roth *et al.* [14]
Chiron	A	13	Intravenous infusion of retroviral vector expressing BDD-FVIII	Powell *et al.* [15]
GenStar	A	1	Helper-dependent adenoviral vector expressing full-length FVIII under control of albumin promoter	White [48]
CHOP, Stanford, Avigen	B	8	Direct intramuscular injection of AAV2 expressing FIX under control of CMV promoter	Kay *et al.*, Manno *et al.* [57,58]
Stanford, CHOP, Avigen	B	7	Hepatic artery injection of AAV2 expressing FIX under control of hAAT promoter	Manno *et al.* [2]
CHOP	B	Initiated	Hepatic artery injection of AAV2 expressing FIX under control of hAAT promoter with transient immune modulation	High [74]
St. Judes	B	Planned	Intravascular deliver of AAV8 expressing FIX under control of hAAT promoter; self-complementary genome	Nienhuis [75]

AAV, adeno-associated virus; BDD, B domain deleted; CMV, cytomegalovirus; FIX, factor IX.

subretinal space, or into the subthalamic nucleus in subjects with Parkinson's disease, resulted respectively in statistically significant improvement in objective and psychophysical measures of vision, or of clinical motor scores and brain metabolism as measured by positron emission tomography (PET) scanning. Thus, proof of concept of the general approach of gene transfer is now established and the task is to adapt it successfully for hemophilia.

Initial trials of gene therapy for hemophilia

As with other plasma protein deficiencies, a variety of gene transfer approaches hold the potential to yield success in hemophilia. This is reflected in the range of strategies that were investigated in the initial round of clinical studies.

Plasmid transfection of autologous fibroblasts

The first hemophilia gene therapy trial reviewed and approved by the US Food and Drug Administration took place at the Beth Israel Deaconness Medical Center in Boston. Sponsored by a biotechnology company, Transkaryotic Therapeutics, the study enrolled a total of 12 subjects with severe hemophilia A, for a procedure in which autologous fibroblasts were harvested from a skin biopsy and stably transfected with a plasmid encoding B-domain-deleted factor VIII (FVIII) under the control of the fibronectin promoter. Preclinical studies using the same strategy in mice resulted in human FVIII levels of ~5% maintained for >1 year [14]. For the human study, a single clone was selected and characterized in terms of FVIII expression levels and growth in soft-agar (a tumorigenicity assay). The clone was expanded, and 7 weeks after skin biopsy, either 10^8 or 4×10^8 autologous genetically modified fibroblasts were implanted onto the omentum in a laparoscopic procedure. Results for the first six subjects (three with each of the two cell doses) have been published [14]; the subjects ranged in age from 20 to 72 years (mean age 46); four out of six were human immunodeficiency virus (HIV)-positive but did not have acquired immune deficiency syndrome (AIDS), and six out of six were hepatitis C virus (HCV) antibody-positive. In the higher-dose group, one subject had a marked decrease in the frequency of spontaneous bleeds that lasted for ~10 months; this subject also demonstrated circulating FVIII levels above baseline (in the range of 1–4%) for ~6 months. *In vitro* testing of the transfected fibroblasts also demonstrated a higher level of FVIII expression in this subject's fibroblasts. After ~10 months, this patient's FVIII levels fell to baseline and spontaneous bleeding recurred. The etiology of the loss of expression was not determined, but may have been because of a cellular immune response to the FVIII-expressing cells; gradual senescence of the cells; a fibrotic reaction around the site of implantation that reduced secretion of FVIII into the circulation; transcriptional repression; or some other factor(s). Clearly the procedure, though labor intensive, was safe, and offers several attractive features. Because a single clone is selected and implanted, the risk of insertional mutagenesis is minimized. Because gene transfer takes place *ex vivo*, the risk of an immune response is reduced, and the risk of germline transmission of the donated DNA is also lessened. Based on the clinical and laboratory results in the report, one would conclude that substantially higher levels of expression, or higher cell doses, would be required for a therapeutic effect. Moreover, the levels of FVIII production in tissue culture did not accurately predict the circulating levels once the cells were implanted, likely because transfer into the circulation was <100%. This strategy may come back into play in a modified form, using, for example, genetically modified induced pluripotent stem cells.

Intravenous infusion of a retroviral vector expressing B-domain-deleted factor VIII

In a trial sponsored by the Chiron Corporation, 13 subjects with severe hemophilia A received intravenous infusion of a vesicular stomatitis virus (VSV)-pseudotyped retroviral vector expressing B-domain-deleted FVIII under the control of the viral long terminal repeat (LTR) as promoter [15]. Enrolled subjects ranged in age from 18 to 55 years (mean age 37.5); 5/13 were HIV positive and all were HCV antibody positive. The study had a dose escalation design, with doses ranging from 2.8×10^7 transducing units (TU)/kg up to 8.8×10^8 TU/kg. Infusion proved safe, but there was no evidence for long-term expression of the gene as measured by persistent increases in circulating levels of FVIII. There was evidence by polymerase chain reaction (PCR) for persistence of transgene DNA for at least 1 year in peripheral blood mononuclear cells (PBMCs) of all of the three subjects tested. The trial demonstrated that there were no adverse events associated with intravenous infusion of a retroviral vector at these doses.

The potential for efficacy of this approach was difficult to assess based on the preclinical studies, as they utilized vectors expressing human FVIII in rabbits and hemophilic dogs. (The canine factor VIII gene was not cloned until 1998 [16], and was thus not available when these studies were initiated.) These animals rapidly form antibodies to human FVIII, so that measurement of circulating levels becomes problematic; the studies in hemophilic dogs were published in abstract form [17,18] but not in full-length format, further complicating objective analysis. Certainly, the data suggest that neither rabbits nor dogs accurately predicted dosing in humans; the rabbit studies showed circulating FVIII levels of ~10–20% normal after a total dose of $\sim 4 \times 10^8$ CFU/kg [19], while the hemophilic dogs infused at the same dose showed levels as high as 10% [18].

A characteristic of retroviral vectors is a requirement for dividing target cells, since disruption of the nuclear membrane is necessary for the retroviral preintegration complex to gain access to the chromatin [20,21]. One would thus predict that the most likely targets for retroviral transduction after intravenous infusion would be the rapidly dividing cells in the bone marrow, or perhaps dividing cells in the gastrointestinal (GI) tract. Consistent with this principle, vector DNA was indeed recovered from PBMCs of infused subjects in the clinical trial. Certainly, it would be unlikely to target the liver in an adult animal (or human) without some prior maneuver to induce hepatocyte cell division. Thus, in an earlier study, Kay and colleagues achieved transduction of canine liver with a retroviral vector expressing factor IX by first carrying out partial hepatectomy to induce cell division in the remaining hepatocytes [22]. Similarly, in subsequent studies, two groups, those of VandenDriessche and of Ponder, demonstrated that intravenous infusion of a retroviral vector could result in sustained expression of therapeutic levels of clotting factors if neonatal animals, in which hepatocytes are still rapidly dividing, are the recipients [23,24]. (Even in these studies with neonatal mice and dogs, though, vector doses required were approximately one log higher than the highest doses administered in the clinical trial.) Ponder and colleagues also demonstrated that another maneuver, coadministration of hepatocyte growth factor, could result in some modest level of transduction of liver. Animal studies suggest that without some method to ensure access to dividing hepatocytes, retroviral infusion is not likely to be successful in the setting of hemophilia. However, an advance that resulted from these efforts was the ability to generate higher-titer vectors and resulting potential to administer higher doses.

Subsequent preclinical studies have built on these initial observations to develop approaches with a higher likelihood of efficacy. First, the development of lentiviral vectors, which can transduce a wide variety of target cells including nondividing cells, has widened options for hemophilia and many other disorders. Although clinical use of lentiviral vectors has been quite limited to date [25], several experimental approaches using lentiviral vectors have resulted in phenotypic improvement in small animal models of hemophilia. For example, Poncz and colleagues, and Montgomery *et al*., used both transgenic and lentiviral approaches to deliver the B-domain-deleted FVIII gene to hematopoietic stem cells in mice. Using a megakaryocyte-specific promoter, they demonstrated that platelet FVIII was stored in α-granules and released on platelet activation at the site of an injury [26,27], and that platelet-released FVIII could correct the bleeding phenotype even in the presence of inhibitory antibodies [28], although the degree of efficacy in this setting is still debated [29]. In a similar approach, Sadelain and colleagues have used a lentiviral vector with an erythroid-specific promoter to direct factor IX synthesis in erythroblasts, and this too has shown stable expression of FIX levels in the range of 5–7% in lethally irradiated mice transplanted with transduced bone marrow from syngeneic mice [30]. In both of these approaches, clinical feasibility will likely depend on the ability to achieve success using reduced-intensity conditioning regimens. Sadelain *et al*. have already used such an approach (low-dose busulfan for conditioning), combined with *in vivo* selection for transduced cells using methylguanine methyltransferase (MGMT) to achieve long-term expression of circulating FIX levels of >10% in the hemophilia B mouse model using the erythroid-specific lentiviral vector [31]. The relatively high circulating levels seen with the erythroid approach are associated with tolerance to the FIX protein even in the hemophilic mice, another benefit of this approach. Plans are under way to extend these approaches to large animal models to test for safety and efficacy.

A third approach using lentiviral vectors targets hepatocytes for expression. Early efforts using ubiquitously expressed promoters resulted in immune responses to factor IX or factor VIII that limited expression [32–34]. The use of a liver-specific promoter attenuated but did not entirely extinguish B- and T-cell responses to the transgene product [35,36]. In follow-up

studies, Naldini and coworkers demonstrated that intravenous infusion of lentiviral vectors triggers an interferon response that further limits the efficiency and stability of lentiviral-mediated gene transfer to liver [37]. However, a recent study showed that inclusion of a miRNA target sequence in a lentiviral vector could suppress expression in antigen-presenting cells and further reduce immune responses to the transgene product [38]. Given that immune responses to the transgene product are a serious complication in hemophilia, as they may limit the ability to treat the disease with protein concentrates, it will be important to test the safety and efficacy of this strategy in a large animal model of hemophilia before contemplating human trials. The major safety concern with integrating vectors, the risk of insertional mutagenesis, may be lessened with lentiviral versus retroviral vectors, because of reduced predilection for integration near transcription start sites [39,40].

Infusion of an adenoviral vector expressing factor VIII

Recombinant adenoviruses are double-stranded DNA vectors that are highly efficient for gene transfer efficiency and able to target hepatocytes with high specificity through the coxsackie virus adenovirus receptor (CAR) receptor [41]. They have been used extensively in gene transfer studies of hemophilia in animal models. Studies in mice demonstrated that durable gene transfer could be achieved for both FVIII and FIX and sustained for many months [42,43]. However, only transient transgene expression was observed in initial studies in large animal models because of the strong innate immune responses elicited by the vector. In a canine model of hemophilia B, a total dose of 2.4×10^{12} plaque-forming units (PFU) of Ad-cF.IX resulted in transient supraphysiologic levels of FIX that declined to baseline levels within 2 months, presumably as a result of immune-mediated destruction of transduced hepatocytes [44]. A study in nonhuman primates that received recombinant adenovirus encoding human FIX at doses ranging from 3.4×10^{11} to 3.8×10^{12} vector particles (vp) per kilogram resulted in transient physiologic levels of circulating factor, but this was accompanied by dose-dependent liver and skeletal muscle toxicity, coagulation abnormalities, and decreased platelet counts [45]. These early *in vivo* observations catalyzed further vector optimization efforts to eliminate residual viral coding sequences implicated in the acute inflammatory responses. The resulting high capacity (HC), helper-dependent (HD) adenovirus vectors do not contain any viral genes and were therefore predicted to be less inflammatory compared with the first-generation vectors. A hemophilia A dog injected with a dose of 3×10^{11} vp/kg of HC-Ad containing a canine FVIII expression cassette resulted in a maximum level of 3.5% achieved 3 days following vector administration in the absence of any detectable hepatotoxicity, nephrotoxicity, or hematologic abnormalities [46]. However, the level of circulating factor returned to baseline levels (<1%) by 14 days post-vector infusion. Administration of HC-Ad encoding human FVIII in nonhuman primates revealed attenuated, dose-dependent toxicity, with no toxicity observed at 1.4×10^{12} vp/kg, but significant hepatotoxicity at 4.3×10^{12} vp/kg [47].

In a phase I clinical trial for hemophilia A sponsored by the biotechnology company GenStar Therapeutics, a single subject with severe hemophilia A was infused with 4.3×10^{10} vp/kg of recombinant HC-Ad containing the full length cDNA of human FVIII under the control of the human albumin promoter [48]. This dose was 100-fold lower than the dose found to cause hepatoxicity in nonhuman primates [47]. The subject experienced fever and chills ~5 h after vector infusion, and thrombocytopenia and elevation in liver enzymes that peaked ~7 days following infusion. These symptoms resolved within 19 days [49]. Circulating FVIII levels of 1% were reported for several months, but a causal relationship of vector administration with these low levels (which are close to the baseline of methods used to measure blood FVIII) was unclear. Owing to safety concerns and the narrow potential therapeutic index, indicated by subtherapeutic transgene expression at a dose that resulted in moderate toxicity, combined with the prior occurrence of a fatal acute response in a human subject who received a 14-fold higher dose (6×10^{11} vp/kg) of a different adenovirus vector in a trial for ornithine transcarbamylase (OTC) deficiency [50], no additional subjects were enrolled in this trial.

Innate immune responses to the adenovirus capsid protein resulting in dose-limiting acute toxicity remain a major challenge for adenovirus vector-mediated gene transfer to the liver. Further improvements have been made to enhance the safety and therapeutic potential of recombinant adenovirus for liver-directed gene transfer. In particular, more efficient and localized vector infusion to the liver by balloon occlusion catheter delivery was found to reduce systemic exposure and associated vector toxicity, and result in sustained expression of a reporter transgene [51]. However, the risk associated with administration of recombinant adenovirus relative to their potential benefit likely needs to be reduced to support its further development for clinical applications such as hemophilia for which partially effective therapies have been established.

Intramuscular injection of an adeno-associated virus vector expressing factor IX

Gene transfer vectors based on AAVs have demonstrated promise for human gene therapy based on their excellent safety and long-term efficacy in animal models, and several clinical studies for both genetic and acquired diseases using this vector have been initiated [12,52,53]. The inability to replicate autonomously and lack of any known disease association with wild-type AAV, as well as its low immunogenicity, make vectors based on AAV excellent candidates for gene transfer for hemophilia. The small vector genome capacity of AAV can accommodate FIX cDNA expression cassettes, but not the larger full-length FVIII cDNA. Hence, translational

efforts using AAV have focused on hemophilia B. Strategies to overcome the challenges presented by the much larger FVIII expression cassette have been developed [54,55], and offer promise for future AAV vector-based clinical studies for hemophilia A.

An AAV2-based vector encoding canine FIX under the control of a cytomegalovirus (CMV) promoter was developed and administered by intramuscular injection to five hemophilia B dogs at doses ranging from 1.3×10^{11} to 8.5×10^{12} vp/kg [56]. A dose-dependent partial correction in the activated partial thromboplastin time (aPTT) and levels of circulating cFIX antigen levels up to 70 ng/mL were observed. The therapeutic effects observed were stable for the 10-month duration of the study. Based on these and other results in animal models supporting safety and efficacy, a phase I/II clinical trial was initiated in 1999 by The Children's Hospital of Philadelphia, Stanford University, and the biotechnology company Avigen to test the safety and efficacy of intramuscular delivery of recombinant AAV encoding a FIX transgene cassette with a CMV promoter for hemophilia B. A total of eight subjects with severe hemophilia B received intramuscular injection of vector in three dose cohorts ranging from 2×10^{11} to 1.8×12^{12} vp/kg [57,58]. Administration was well tolerated in all subjects, with no adverse events observed in the immediate post-treatment period, nor during multiyear follow-up. From this study the investigators established that: (i) intramuscular injection of AAV-hF.IX at doses up to 1.8×10^{12} vp/kg was safe and well tolerated; (ii) the level of transduction assessed by vector gene copy number in DNA from muscle biopsies was consistent with that predicted by the preclinical mouse and dog models; and (iii) transgene expression was stable over time, based on muscle biopsies performed up to 3.7 years post administration [59]. However, a problem encountered with this route of administration was the practical limitation of performing the number of injections estimated to be required (~500, based on studies in the canine model) to attain therapeutically significant levels of the donated FIX protein. In addition, concurrent studies showed that gene delivery to skeletal muscle by direct intramuscular injection carried a risk of promoting an immune response to the transgene product FIX [60].

Development of more efficient methods for delivery of AAV to muscle in large animal models was subsequently described. Arruda and colleagues reported long-term robust cFIX expression, corresponding to 4–14% of normal physiologic levels, following AAV vector-mediated gene transfer by regional intravascular delivery to muscle in a canine hemophilia B model using doses comparable to those used in the clinical trial [61]. The approach required a surgical procedure and administration of histamine to allow egress of vector from the vasculature and efficient myocyte transduction. Subsequent development of a more clinically feasible procedure, in which vector is administered in a large volume of fluid into the saphenous vein (so that hydrostatic pressure rather than a vasoactive drug effects vector transfer through the vasculature into myocytes), increases the likelihood that this route of administration can be tested clinically [62]. An advantage of using muscle as a target tissue is that it could provide a therapeutic option for individuals with severe liver disease.

Liver infusion of an adeno-associated virus vector expressing factor IX

Compared with intramuscular injection, higher circulating FIX levels have been observed in animal models following liver transduction, likely owing to more efficient secretion of FIX into the circulation from hepatocytes. Mount and colleagues reported that a dose of 1×10^{12} vp/kg resulted in canine FIX levels ranging from 5% to 12% of normal values in hemophilia B dogs, achieving almost complete correction of the hemophilia phenotype [3]. Nathwani and colleagues administered a dose of 4×10^{12} vp/kg in rhesus macaques and measured FIX levels ranging from 0.8% to 10% [63]. In this study one animal developed a FIX inhibitor, likely attributable to the amino acid differences between the human and macaque FIX sequences. Together, these animal model studies demonstrated the ability of AAV2-FIX to transduce liver cells, resulting in durable and clinically significant circulating FIX levels without detectable toxicity.

The promising results of liver-directed gene transfer in animal models prompted a second hemophilia B clinical trial sponsored by Stanford University, Children's Hospital of Philadelphia, and Avigen [2] to investigate an optimized AAV2 vector containing an expression cassette for FIX under the control of a liver-specific human α_1-antitrypsin promoter. In the initial phase of the study, a total of seven subjects with severe hemophilia B received vector via hepatic artery infusion in three dose cohorts ranging from 8×10^{10} to 2×10^{12} vp/kg. Following administration at the low and mid doses, no FIX expression was detected. However, in the first subject treated at the high dose (subject E), clinically significant levels of FIX were detected in the blood. Factor IX was undetectable prior to vector administration, and rose to a peak of 11.2% 2 weeks after vector infusion, a level accurately predicted by the preclinical animal studies [3]. Subject E had no detectable preexisting antibodies to AAV2. Therapeutic levels of FIX were measured in this subject for approximately 5 weeks, but then returned to baseline concomitant with a transient, self-limiting, asymptomatic transaminitis, and the appearance in the peripheral blood of a population of capsid-specific $CD8^+$ T cells [2,64]. In the second subject that received the high dose, but who had a pre-existing anti-AAV2 titer of 1:17, neither transgene expression nor transient liver enzyme elevation were observed. In one additional subject with a low preexisting AAV2 antibody titer who subsequently received the mid-dose (4×10^{11} vp/kg), a mild, self-limiting transaminitis that recapitulated the temporal pattern observed in subject E, but with reduced amplitude, was observed.

The observations made during the AAV-FIX liver-directed clinical trial confirmed that recombinant AAV2 was capable

of achieving clinically significant levels of FIX following liver infusion in human subjects. However, barriers relating to human immune responses were revealed. It became clear that the presence of even modest levels (i.e., 1:17) of pre-existing AAV antibodies was sufficient to block transduction using this route of administration (although not with intramuscular injection). Unexpectedly, because not observed in any animal models, an adaptive immune response, likely a memory response primed by a preceding exposure to infection by the ubiquitous wild-type AAV2 virus, led to an AAV capsid-specific cytotoxic T-lymphocyte (CTL)-mediated destruction of transduced hepatocytes. The etiology of this efficacy-limiting immune response was the topic of an NIH Recombinant DNA Advisory Committee Symposium convened in 2007 [65]. Hypotheses that were advanced to explain the provenance of the capsid peptides that were presented by major histocompatibility complex (MHC) class I molecules on transduced human hepatocytes, thereby sensitizing them to capsid-specific CTL effector function, included: (i) residual *cap* DNA introduced into the vector-transduced cells which was transcribed, translated, and presented [66,67]; (ii) the capsid protein component of the vector inoculum, i.e., presentation of exogenous antigen [68]; and (iii) a cryptic alternative open-reading frame in the clinical transgene cassette sequence which was transcribed, translated, and presented [65]. (This latter hypothesis assumes that the documented $CD8^+$ T-cell response to the capsid was unrelated, and that the true antigen had been missed.) Sensitive transcriptional profiling studies using the actual vector used in the clinical study excluded the possibility that trace *cap* DNA expression accounted for sensitization of vector-transduced hepatocytes, and subsequent studies supported the second hypothesis, that processing and MHC class I presentation of peptides directly derived from the preformed capsid protein component of the vector inoculum occurred [68,69]. Together, these studies revealed the potential for mild, but efficacy-limiting immunotoxicity attributable to the capsid protein component of the AAV vector following human liver administration. The results support that the amount of AAV vector capsid protein used in vector prepared for clinical studies should be the lowest level required to achieve delivery of an efficacious dose of therapeutic transgene [70,71]. Strategies to achieve this objective include the use of highly efficient vectors [72], combined with rigorous attention to detail of the preparation of this novel class of biologic product for use in clinical studies to minimize their potential immunogenicity [73].

The first compelling evidence of efficacy following gene transfer for hemophilia was documented in the liver-directed AAV-FIX hemophilia B trial. This result, combined with the now more complete understanding of immune barriers to AAV vector-mediated long-term therapeutic gene transfer in human subjects have moved the field closer to truly effective gene transfer for hemophilia. The recent successful treatment of Leber Congenital Amaurosis [10–12] and Parkinson's disease [13], in which low doses of AAV vector are delivered to relatively immunoprivileged sites, further underscores the potential if the immune responses that arise in other routes of administration can be controlled. One strategy that is currently being tested in the clinic is the use of transient immune modulation to block the activation and proliferation of AAV capsid-specific CTLs that were implicated in limiting efficacy until the capsid antigen has been degraded and disappears from transduced cells. A second strategy that will soon be tested in the clinic is the use of a more efficient serotype (AAV8) and more efficient, self-complementary FIX expression cassette that is expected to dramatically reduce the vector dose required to achieve clinically significant levels of circulating FIX [72]. The lessons learned in the last 10 years of clinical gene transfer may eventually translate into improved therapies for hemophilia and other genetic disorders.

References

1 Murphy SL, High KA. Gene therapy for haemophilia. *Br J Haematol* 2008; **140**: 479–87.
2 Manno CS, Pierce GF, Arruda VR, *et al*. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med* 2006; **12**: 342–7.
3 Mount JD, Herzog RW, Tillson DM, *et al*. Sustained phenotypic correction of hemophilia B dogs with a factor IX null mutation by liver-directed gene therapy. *Blood* 2002; **15**: 2670–6.
4 Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, *et al*. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000; **288**: 669–72.
5 Hacein-Bey-Abina S, von Kalle C, Schmidt M, *et al*. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 2003; **348**: 255–6.
6 Howe SJ, Mansour MR, Schwarzwaelder K, *et al*. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J Clin Invest* 2008; **118**: 3143–50.
7 Aiuti A, Cattaneo F, Galimberti S, *et al*. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *N Engl J Med* 2009; **360**: 447–58.
8 Aiuti A, Slavin S, Aker M, *et al*. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science* 2002; **296**: 2410–13.
9 Mavilio F, Pellegrini G, Ferrari S, *et al*. Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. *Nat Med* 2006; **12**: 1397–1402.
10 Bainbridge JW, Smith AJ, Barker SS, *et al*. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med* 2008; **358**: 2231–9.
11 Hauswirth WW, Aleman TS, Kaushal S, *et al*. Treatment of Leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. *Hum Gene Ther* 2008; **19**: 979–90.
12 Maguire AM, Simonelli F, Pierce EA, *et al*. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med* 2008; **358**: 2240–8.
13 Kaplitt MG, Feigin A, Tang C, *et al*. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet* 2007; **369**: 2097–105.

14 Roth DA, Tawa NE, Jr., O'Brien JM, *et al*. Nonviral transfer of the gene encoding coagulation factor VIII in patients with severe hemophilia A. *N Engl J Med* 2001; **344**: 1735–42.
15 Powell JS, Ragni MV, White GC, 2nd, *et al*. Phase 1 trial of FVIII gene transfer for severe hemophilia A using a retroviral construct administered by peripheral intravenous infusion. *Blood* 2003; **102**: 2038–45.
16 Cameron C, Notley C, Hoyle S, *et al*. The canine factor VIII cDNA and 5′ flanking sequence. *Thromb Haemost* 1998; **79**: 317–22.
17 Greengard JS, Bodner M, McCormack J, *et al*. Systemic expression of human factor VIII from peripheral retroviral delivery in rabbits sand dogs. *Blood* 1997; **90**(Suppl. 1): 240a.
18 Lothrop C, Niemeyer GP, Dufresne M, *et al*. Treatment of canine hemophilia A by direct infusion of retroviral vector expressing the human FVIII cDNA. *Mol Ther* 2000; *5*: S289 (abstract).
19 Greengard JS, Jolly DJ. Animal testing of retroviral-mediated gene therapy for factor VIII deficiency. *Thromb Haemost* 1999; **82**: 555–61.
20 Miller DG, Adam MA, Miller AD. Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. *Mol Cell Biol* 1990; **10**: 4239–42.
21 Roe T, Reynolds TC, Yu G, *et al*. Integration of murine leukemia virus DNA depends on mitosis. *EMBO J* 1993; **12**: 2099–108.
22 Kay MA, Rothenberg S, Landen CN, *et al*. In vivo gene therapy of hemophilia B: sustained partial correction in factor IX-deficient dogs. *Science* 1993; **262**: 117–119.
23 VandenDriessche T, Vanslembrouck V, Goovaerts I, *et al*. Long-term expression of human coagulation factor VIII and correction of hemophilia A after in vivo retroviral gene transfer in factor VIII-deficient mice. *Proc Natl Acad Sci USA* 1999; **96**: 10379–84.
24 Xu L, Gao C, Sands MS, Cai SR, *et al*. Neonatal or hepatocyte growth factor-potentiated adult gene therapy with a retroviral vector results in therapeutic levels of canine factor IX for hemophilia B. *Blood* 2003; **101**: 3924–32.
25 Levine BL, Humeau LM, Boyer J, *et al*. Gene transfer in humans using a conditionally replicating lentiviral vector. *Proc Natl Acad Sci USA* 2006; **103**: 17372–7.
26 Yarovoi HV, Kufrin D, Eslin DE, *et al*. Factor VIII ectopically expressed in platelets: efficacy in hemophilia A treatment. *Blood* 2003; **102**: 4006–13.
27 Shi Q, Wilcox DA, Fahs SA, *et al*. Lentivirus-mediated platelet-derived factor VIII gene therapy in murine haemophilia A. *J Thromb Haemost* 2007; **5**: 352–61.
28 Shi Q, Wilcox DA, Fahs SA, *et al*. Factor VIII ectopically targeted to platelets is therapeutic in hemophilia A with high-titer inhibitory antibodies. *J Clin Invest* 2006; **116**: 1974–82.
29 Gewirtz J, Thornton MA, Rauova L, *et al*. Platelet-delivered factor VIII provides limited resistance to anti-factor VIII inhibitors. *J Thromb Haemost* 2008; **6**: 1160–6.
30 Chang AH, Stephan MT, Sadelain M. Stem cell-derived erythroid cells mediate long-term systemic protein delivery. *Nat Biotechnol* 2006; **24**: 1017–21.
31 Chang AH, Stephan MT, Lisowski L, *et al*. Erythroid-specific human factor IX delivery from *in vivo* selected hematopoietic stem cells following nonmyeloablative conditioning in hemophilia B mice. *Mol Ther* 2008; **16**: 1745–52.
32 Kootstra NA, Matsumura R, Verma IM. Efficient production of human FVIII in hemophilic mice using lentiviral vectors. *Mol Ther* 2003; **5**: 623–31.
33 Park F, Ohashi K, Kay MA. Therapeutic levels of human factor VIII and IX using HIV-1-based lentiviral vectors in mouse liver. *Blood* 2000; **96**: 1173–6.
34 Tsui LV, Kelly M, Zayek N, *et al*. Production of human clotting Factor IX without toxicity in mice after vascular delivery of a lentiviral vector. *Nat Biotechnol* 2002; **20**: 53–7.
35 Brown BD, Shi CX, Rawle FE, *et al*. Factors influencing therapeutic efficacy and the host immune response to helper-dependent adenoviral gene therapy in hemophilia A mice. *J Thromb Haemost* 2004; **2**: 111–18.
36 Follenzi A, Battaglia M, Lombardo A, *et al*. Targeting lentiviral vector expression to hepatocytes limits transgene-specific immune response and establishes long-term expression of human antihemophilic factor IX in mice. *Blood* 2004; **103**: 3700–9.
37 Brown BD, Cantore A, Annoni A, *et al*. A microRNA-regulated lentiviral vector mediates stable correction of hemophilia B mice. *Blood* 2007; **110**: 4144–52.
38 Brown BD, Venneri MA, Zingale A, *et al*. Endogenous microRNA regulation suppresses transgene expression in hematopoietic lineages and enables stable gene transfer. *Nat Med* 2006; **12**: 585–91.
39 Schroder AR, Shinn P, Chen H, *et al*. HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* 2002; **110**: 521–9.
40 Wu X, Li Y, Crise B, *et al*. Transcription start regions in the human genome are favored targets for MLV integration. *Science* 2003; **300**: 1749–51.
41 Bergelson JM, Cunningham JA, Droguett G, *et al*. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science* 1997; **275**: 1320–3.
42 Connelly S, Gardner JM, Lyons RM, *et al*. Sustained expression of therapeutic levels of human factor VIII in mice. *Blood* 1996; **87**: 4671–7.
43 Walter J, You Q, Hagstrom JN, *et al*. Successful expression of human factor IX following repeat administration of adenoviral vector in mice. *Proc Natl Acad Sci USA* 1996; **93**: 3056–61.
44 Kay MA, Landen CN, Rothenberg SR, *et al*. In vivo hepatic gene therapy: complete albeit transient correction of factor IX deficiency in hemophilia B dogs. *Proc Natl Acad Sci USA* 1994; **91**: 2353–7.
45 Lozier JN, Metzger ME, Donahue RE, *et al*. The rhesus macaque as an animal model for hemophilia B gene therapy. *Blood* 1999; **93**: 1875–81.
46 Chuah MK, Schiedner G, Thorrez L, *et al*. Therapeutic factor VIII levels and negligible toxicity in mouse and dog models of hemophilia A following gene therapy with high-capacity adenoviral vectors. *Blood* 2003; **101**: 1734–43.
47 Fang B, Andreason G, Hariharan M, *et al*. Pre-clinical efficacy and safety studies of a gutless adenovirus vector (MAXADFVIII) for treatment of haemophilia A. *Thromb Haemost* 2001; **86**: OC2490 (abstract).
48 White GC, 2nd. Gene therapy in hemophilia: clinical trials update. *Thromb Haemost* 2001; **86**: 172–7.
49 Chuah MK, Collen D, VandenDriessche T. Clinical gene transfer studies for hemophilia A. *Sem Thromb Hemost* 2004; **30**: 249–56.
50 Knorr DW. Minutes of Symposium and Meeting; Recombinant DNA Advisory Committee, December 8–10, 1999. *Hum Gen Ther* 2000; **11**: 1591–621.
51 Brunetti-Pierri N, Stapleton GE, Law M, *et al*. Efficient, long-term hepatic gene transfer using clinically relevant HDAd doses by balloon occlusion catheter delivery in nonhuman primates. *Mol Ther* 2009; **17**: 327–33.
52 Carter BJ. Adeno-associated virus vectors in clinical trials. *Hum Gene Ther* 2005; **16**: 541–50.

53 Warrington KH, Jr., Herzog RW. Treatment of human disease by adeno-associated viral gene transfer. *Hum Gen* 2006; **119**: 571–603.
54 Scallan CD, Liu T, Parker AE, *et al*. Phenotypic correction of a mouse model of hemophilia A using AAV2 vectors encoding the heavy and light chains of FVIII. *Blood* 2003; **102**: 3919–26.
55 Jiang H, Lillicrap D, Patarroyo-White S, *et al*. Multiyear therapeutic benefit of AAV serotypes 2, 6, and 8 delivering factor VIII to hemophilia A mice and dogs. *Blood* 2006; **108**: 107–15.
56 Herzog RW, Yang EY, Couto LB, *et al*. Long-term correction of canine hemophilia B by gene transfer of blood coagulation factor IX mediated by adeno-associated viral vector. *Nat Med* 1999; **5**: 56–63.
57 Kay MA, Manno CS, Ragni MV, *et al*. Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. *Nat Genet* 2000; **24**: 257–61.
58 Manno CS, Chew AJ, Hutchison S, *et al*. AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B. *Blood* 2003; **101**: 2963–72.
59 Jiang H, Pierce GF, Ozelo MC, *et al*. Evidence of multiyear factor IX expression by AAV-mediated gene transfer to skeletal muscle in an individual with severe hemophilia B. *Mol Ther* 2006; **14**: 452–5.
60 Herzog RW, Fields PA, Arruda VR, *et al*. Influence of vector dose on factor IX-specific T and B cell responses in muscle-directed gene therapy. *Hum Gene Ther* 2002; **13**: 1281–91.
61 Arruda VR, Stedman HH, Nichols TC, *et al*. Regional intravascular delivery of AAV-2-F.IX to skeletal muscle achieves long-term correction of hemophilia B in a large animal model. *Blood* 2005; **105**: 3458–64.
62 Su LT, Gopal K, Wang Z, *et al*. Uniform scale-independent gene transfer to striated muscle after transvenular extravasation of vector. *Circulation* 2005; **112**: 1780–8.
63 Nathwani AC, Davidoff AM, Hanawa H, *et al*. Sustained high-level expression of human factor IX (hFIX) after liver-targeted delivery of recombinant adeno-associated virus encoding the hFIX gene in rhesus macaques. *Blood* 2002; **100**: 1662–9.
64 Mingozzi F, Maus MV, Hui DJ, *et al*. CD8(+) T-cell responses to adeno-associated virus capsid in humans. *Nat Med* 2007; **13**: 419–22.
65 http://www4.od.nih.gov/oba/RAC/meeting.html. NIH Recombinant DNA Advisory Committee, AAV Symposium. Immune Response to Adeno-Associated Virus (AAV) Vectors, Bethesda, MD, 2007.
66 Gao G, Wang Q, Wang L, *et al*. Inadvertent gene transfer of co-packaged Rep and Cap sequences during the production of AAV vector and its potential impact on vector performance. *Mol Ther* 2008; **16**: S105.
67 Nony P, Chadeuf G, Tessier J, *et al*. Evidence for packaging of rep-cap sequences into adeno-associated virus (AAV) type 2 capsids in the absence of inverted terminal repeats: a model for generation of rep-positive AAV particles. *J Virol* 2003; **77**: 776–81.
68 Pien GC, Basner-Tschakarjan E, Hui DJ, *et al*. Capsid antigen presentation flags hepatocytes for destruction after transduction by adeno-associated viral vectors. *J Clin Invest* 2009; **119**: 1688–95.
69 Hauck B, Murphy SL, Smith PH, *et al*. Undetectable transcription of cap in a clinical AAV vector: implications for preformed capsid in immune responses. *Mol Ther* 2009; **17**: 144–52.
70 Grimm D, Kern A, Pawlita M, *et al*. Titration of AAV-2 particles via a novel capsid ELISA: packaging of genomes can limit production of recombinant AAV-2. *Gene Ther* 1999; **6**: 1322–30.
71 Sommer JM, Smith PH, Parthasarathy S, *et al*. Quantification of adeno-associated virus particles and empty capsids by optical density measurement. *Mol Ther* 2003; **7**: 122–8.
72 Nathwani AC, Gray JT, McIntosh J, *et al*. Safe and efficient transduction of the liver after peripheral vein infusion of self-complementary AAV vector results in stable therapeutic expression of human FIX in nonhuman primates. *Blood* 2007; **109**: 1414–21.
73 Wright JF. Manufacturing and characterizing AAV-based vectors for use in clinical studies. *Gene Ther* 2008; **15**: 840–8.
74 http://oba.od.nih.gov/oba/RAC/meetings/Dec2007/final_RAC_agenda_dec07.pdf. NIH Recombinant DNA Advisory Committee. Presentation by High, KA and vanDeventer, S: Use of immunosuppression with AAV vectors, Bethesda, MD, 2007.
75 http://oba.od.nih.gov/oba/RAC/meetings/Dec2007/final_RAC_agenda_dec07.pdf. NIH Recombinant DNA Advisory Committee. Presentation by Nienhuis AW: An open label dose-escalation study of a self-complementary adeno-associated viral vector (scAAV2/8-LP1-hFIXco) of gene therapy of hemophilia B, Bethesda, MD, 2007.

37 Gene therapy: molecular engineering of factor VIII and factor IX

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Introduction

Insights from recombinant DNA technology ushered in the gene therapy era. As the severe complications of hemophilia can be avoided with even a modest increase in plasma levels of factor VIII (FVIII) and factor IX (FIX), hemophilia became a popular target of gene therapy research. Most current gene therapy strategies rely on gene methodologies using vectors as delivery vehicles to provide a wild-type copy of the defective gene to a physiologically relevant target tissue. The most efficient vectors have proven to be engineered viruses, with known mechanisms for entering eukaryotic cells and harnessing their synthetic machinery to produce foreign proteins. Whereas this application to a wide range of preclinical animal models has demonstrated success for these strategies, there have been significant obstacles remaining for human clinical applications depending on the combination of vector used, expressed transgene and target tissue chosen (see Chapter 36). This has resulted in only a few patients achieving transient low-level expression of either FVIII or FIX.

Continued advances in recombinant DNA technology remains a promising platform to address the remaining challenges for affordable and accessible recombinant clotting factors as well as gene therapy efforts. In particular, ongoing research has provided detailed structure and function characterizations for each phase of the life cycles for FVIII (Figure 37.1) and FIX: biosynthesis, macromolecular interactions, activation/inactivation, and clearance. This has come through insights from the study of hemophilia mutations, site-directed mutagenesis, detailed structural models and an expanded repertoire of animal models through molecular biology advances. This has opened up new frontiers for bioengineering strategies to overcome some of the remaining limitations inherent to current clotting factor concentrates. Partnering some of these bioengineering strategies with advances in gene therapy, vectorology and immunology insights may increase the likelihood of a successful and broadly applicable gene therapy strategy.

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Factor VIII with improved functional properties (Table 37.1)

Improved biosynthesis and secretion

Several mechanisms have been identified that limit FVIII expression: (i) FVIII is susceptible to misfolding within the endoplasmic reticulum (ER), inducing transcription of ER stress-response genes through the unfolded protein response (UPR), a cellular adaptive response to regulate all levels of gene expression including transcription, translation, translocation into the ER lumen and ER-associated degradation [1]; and (ii) FVIII forms nondisulfide-bonded high-molecular-weight aggregates that are retained within the ER through interactions with the protein chaperones immunoglobulin binding protein (BiP/GRP78), calnexin and calreticulin. FVIII also demonstrates a requirement for facilitated transport from the ER to the Golgi apparatus through interaction with the lectin LMAN1–MCFD2 complex. Although the study of FVIII synthesis and secretion in heterologous expression systems has its limitations, this is the method by which recombinant FVIII (rFVIII) is produced, and some gene therapy strategies are presently directed to express FVIII in cells that do not normally produce the protein [2].

Increased mRNA expression

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 as a result of markedly increased levels of mRNA and increased translation [3,4]. The smaller size of the B-domain-deleted (BDD)-FVIII cDNA facilitated packaging within certain viral

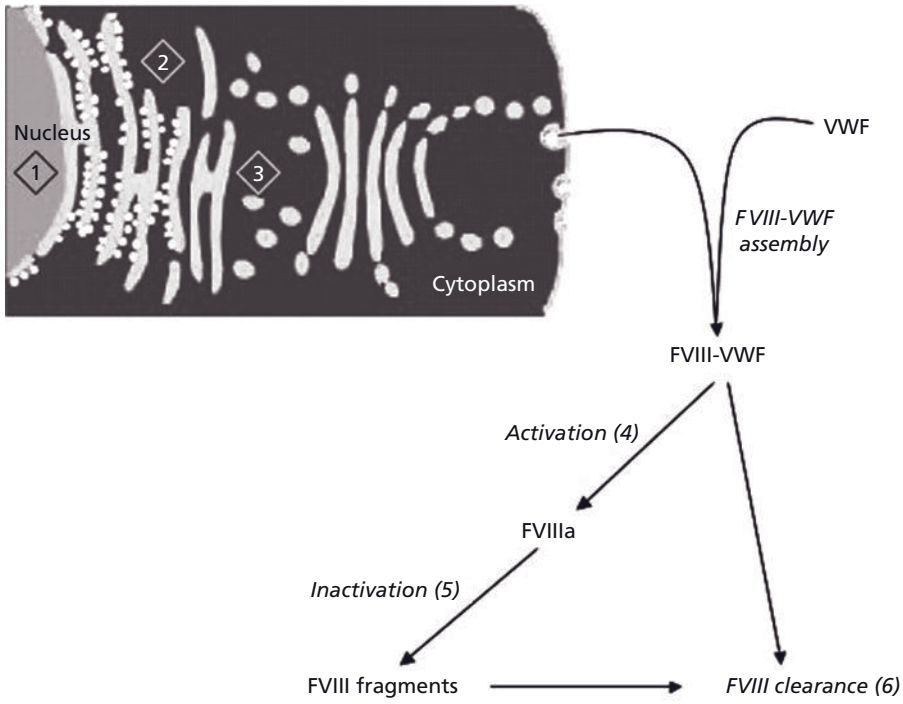

Figure 37.1 Overview of the factor VIII life cycle and targets for bioengineering strategies. Schematic of factor VIII biosynthesis from mRNA expression (1), chaperone-assisted folding with the ER (2), facilitate transport through the ER–Golgi intermediate compartment (3), secretion and assembly with VWF, activation (4), inactivation (5), and clearance from plasma (6). Numbers indicate realized targets of bioengineering.

Table 37.1 Summary of bioengineering strategies for FVIII with improved functional properties.

Targeted functional improvement	Bioengineering strategy	References
Improved biosynthesis and secretion		
Increased mRNA expression	Removal of entire B domain	3, 4
	Substitution with FIX intron 1	10
Reduced ER chaperone interactions		18
Improved ER-Golgi transport and reduced ER stress		28
Other		33, 34
Improved functional activity		
Increaased activation	des-(868–1562)-FVIII–HCII	37
Resistance to inactivation	Inactivation-resistant FVIII (IR8) (Arg336IIe/Arg562Lys/Arg740Ala/ des-794–1689)	38, 39
	A2–A3 disulfide-bridged FVIII (Cys664–Cys1826)	41
	Alteration of charged amino acids at A domain interfaces (Ala/Val substitution at Glu272, Asp519, Glu665, Glu1984)	42, 43
Improved plasma half-life		
	LRP binding site mutations (A2 domain residues 484–509 and C2 domain)	44, 45
	Heparin sulfate proteoglycans-binding site mutations (A2 domain residues 558–556)	44, 45

ER, endoplasmic reticulum.

vectors that could not accommodate a full-length FVIII cDNA. Because BDD-FVIII has a biochemical profile similar to wild-type FVIII [4,5], there was enthusiasm to adopt it for gene therapy strategies. Clinical studies have shown the clinical efficacy of BDD-FVIII [6], and rates of inhibitor formation in previously untreated patients with hemophilia A were similar to that observed with full-length rFVIII concentrates [7]. Thus, despite such a major modification of the FVIII protein, this bioengineered form of FVIII was not believed to likely be more immunogenic than wild-type FVIII. Although BDD-FVIII has been successfully expressed in several preclinical gene transfer applications in animal models, expression in human gene therapy applications has still been inefficient [8,9].

Introduction of a truncated intron 1 sequence of FIX within BDD-FVIII cDNA in place of introns 1 and 13 led to a 13-fold increase in FVIII secretion that was associated with a dramati-

cally higher level of FVIII mRNA accumulation in the cell [10]. Intron 13 in the FVIII gene was previously shown to code for part of a transcriptional silencer, which represses expression of FVIII [11]. This truncated intron 1 sequence of FIX has since been incorporated into gene transfer vectors that utilize BDD-FVIII cDNA with therapeutic FVIII plasma levels achieved within mice [12].

Reduced endoplasmic reticulum chaperone interactions

Secretion of FVIII requires its dissociation from the protein chaperone BiP, which resides in the ER and controls the transport of the FVIII primary translation product to the Golgi compartment by retaining misfolded protein molecules in the ER [13,14]. BiP possesses a peptide-stimulated ATPase activity. FVIII release from BiP and transport out of the ER requires high levels of intracellular adenosine triphosphate (ATP) and ATP hydrolysis by BiP [15,16]. A 110-amino acid region within the A1 domain of FVIII was identified, which inhibited its secretion [17]. A putative BiP-binding site was localized to a hydrophobic β-sheet within this 110-amino-acid region, in which 7 of 11 amino acid residues are Leu or Phe. Factor V (FV) has the identical domain structure to FVIII, and these proteins share ~40% amino acid identity between their respective A and C domains. Curiously, FV is 20-fold more efficiently expressed compared with FVIII in mammalian expression systems, and does not interact with BiP. When the 110-amino-acid region of FVIII bordered by residues 226–336 was replaced for the homologous residues from FV, the FV/FVIII hybrid exhibited a fivefold increase in the efficiency of secretion, most likely because of a decreased binding to BiP. However, the generated FV–FVIII hybrid did not retain a cofactor function. Further site-directed mutagenesis within the hydrophobic β-sheet within this 110-amino-acid region of the FVIII A1 domain demonstrated that a single mutation of Phe309 to Ala for the homologous residue from FV (Ser) enhanced secretion of FVIII by threefold and the Phe309Ser mutant retained full cofactor function [18].

Improved endoplasmic reticulum–Golgi transport

The BDD-rFVIII modification produced a 20-fold increase in mRNA levels and expression of FVIII primary translation product [4,19]. However, despite this dramatic difference in accumulation of mRNA, secretion of BDD-rFVIII from the cell was only observed to be approximately twofold higher compared with full-length wild-type FVIII[19]. This was surprising and suggested that the rate of ER–Golgi transport for the BDD-rFVIII molecule was actually reduced compared with FVIII retaining the B domain. More insight into this followed the characterization of the molecular defect underlying the majority of patients with combined deficiency of FV and FVIII. First described in 1954 [20], patients with this rare, autosomal recessive bleeding disorder have plasma levels of FV and FVIII (both antigen and activity) in the range of 5–30 U/dL. The disease-causing mutations have been limited to mutations in two proteins: LMAN1 [also known as ER–Golgi intermediate compartment (ERGIC)–53] and MCFD2 (multiple coagulation factor deficiency 2). LMAN1 is a homohexameric type 1 transmembrane protein. It was characterized as a mannose-binding lectin proposed to target specific glycoproteins to COP-II coated vesicles budding from the ER for transport to the Golgi [21]. Two-thirds of patients described with combined deficiency of FVIII and FV have null mutations of LMAN1, except for a single mutation that disrupts disulfide bond formation critical for its oligomerization [22]. LMAN1 has an N-terminal carbohydrate recognition domain (CRD) with a lectin-type fold consistent with a role in binding glycoproteins. Previous work demonstrated that LMAN1 is required for efficient secretion of FVIII and FV and that this is mediated by oligosaccharide structures within their respective B domains [23]. Almost another one-third of patients can be attributed to mutation in MCFD2, a small soluble protein with two calcium-binding EF-hand motifs in the C-terminus, and interacts directly with LMAN1 to form a 1:1 stoichiometric complex [24,25]. Studies have demonstrated that proper folding of MCFD2 is dependent on the binding of Ca^{2+} to the EF-hand motifs and that its localization to the ERGIC is dependent on its ability to complex to LMAN1 [26]. Interestingly, an MCFD2 mutant that does not complex with LMAN1 retains the ability to bind FVIII [27]. Thus, MCFD2 may be required to facilitate interaction of FVIII and FV with the CRD of LMAN1.

These insights on facilitated transport of FVIII within the secretion pathway have led to reconsideration of the functional role of the B domain and suggests a mechanism for the observed impaired rate of secretion with BDD-FVIII. Recent work has set out to determine the optimal oligosaccharide content required for efficient rate of secretion. When as few as 226 amino acid residues (encoding six potential asparagine-linked glycosylation sites) from the N-terminal portion of the B domain was added to a BDD-FVIII construct, this B-domain variant (226/N6) retained equal efficiency to BDD-FVIII in synthesis of the primary translation product (indicating efficient mRNA accumulation) yet secretion of this rFVIII protein was increased approximately 10-fold compared with BDD-FVIII (consistent with improved efficiency of ER–Golgi transport) [28]. Further, when this B-domain variant was combined with the Phe309Ser mutation, there was a further increase in the efficiency of secretion. This combined construct was then expressed *in vivo* utilizing hydrodynamic tail vein injection of naked plasmid DNA into the FVIII Exon 16 knockout hemophilia A mouse model where it exhibited up to 25-fold higher expression compared with BDD-FVIII. The 226/N6 variant was next delivered into the murine hemophilia A model by a helper-dependent adenoviral vector and resulted in 100% correction of plasma FVIII levels, up to fivefold higher than observed with FVIII WT [29]. Significantly, the 226/N6 variant remained therapeutically efficacious even after a 50% reduction of the viral vector dose. This may be critical to

clinical applications where host responses to the viral vector have limited durable expression *in vivo*. FVIII bioengineered for improved secretion will significantly increase the potential for success in gene therapy strategies for hemophilia A.

Reduced endoplasmic reticulum stress

The effect of FVIII expression in hepatocytes *in vivo* was studied by hydrodynamic tail vein injection of FVIII DNA expression vectors into mice. Injection of the cDNA for either FVIII WT or BDD-FVIII resulted in low but detectable plasma levels of FVIII activity. In contrast, the 226/N6 FVIII B-domain variant resulted in eightfold higher plasma levels of activity and antigen with significantly less intracellular accumulation. In addition, FVIII WT and BDD-FVIII caused oxidative stress, activated the UPR and induced apoptosis *in vivo*. This was not observed for the 226/N6 variant or empty vector, suggesting that more efficient secretion correlates with reduced ER stress [30]. This has important implications for gene therapy applications. A limited study with adenovirus suggested that hemophilia gene therapy may be limited by inflammatory responses associated with the administration of the recombinant adenoviral vector [31]. FVIII that has a tendency to accumulate intracellularly (as seen with FVIII WT and BDD-FVIII) may exacerbate such inflammatory responses and even stimulate production of anti-FVIII antibodies. Consistent with this, within the helper-dependent adenovirus study by Cerullo *et al*., the 226/N6 variant resulted in phenotypic correction for 74 weeks with a low anti-FVIII antibody titer [29]. In another strategy, the 226/N6 variant was incorporated into a nonviral plasmid gene therapy vector and injected into hemophilia A mouse model by hydrodynamic tail vein injection [32]. In control mice treated with BDD-FVIII, FVIII expression dropped to undetectable levels at 2 weeks post injection and high-titer anti-FVIII antibodies were generated in all the plasmid-treated mice. However, in mice treated with the 226/N6 variant, one out of five mice never developed inhibitory antibodies and still had some FVIII gene expression (~10%) at 8 weeks post gene transfer. Three 226/N6 plasmid-treated mice developed anti-FVIII antibodies with significantly reduced inhibitor titer and only one mouse developed high-titer inhibitory antibodies. In addition, the $CD4^+$ T cells isolated from the spleen of mice injected with 226/N6 constructs proliferated less in response to FVIII stimulation than those from mice injected with BDD-FVIII indicating that, under such gene transfer conditions, the 226/N6 protein is less immunogenic than BDD-FVIII.

Other improvements in translation or post-translational processing and transport

During the development of porcine rFVIII for use in patients with inhibitors to human FVIII, it was demonstrated that recombinant porcine BDD-FVIII generated 10–14-fold higher expression than human BDD-FVIII when expressed in baby hamster kidney cell lines [33]. Further, the higher protein production was not caused by significant increases in steady-state FVIII mRNA levels. This suggests a translational or post-translational advantage for this form of porcine rFVIII. Further study using porcine/human hybrids was able to delineate that porcine sequences within the A1 and A3 domains conferred the secretion advantage and that the secretion advantage occurs post-translationally during passage through the ER and Golgi [34]. It was hypothesized that differences in glycosylation between porcine and human factor VIII could contribute to the rate of secretion. BDD-porcine FVIII or porcine–human hybrid constructs could also improve the efficiency of gene therapy applications since improved expression would not require an improvement in gene transfer strategy using currently available viral vector systems. In one application of such a bioengineered FVIII construct, transplantation of hematopoietic stem cells (HSCs) transduced with recombinant retroviruses containing BDD porcine FVIII sequences provided curative FVIII levels in a hemophilia A mouse model [35]. Of particular interest, a similar gene transfer strategy was tested in hemophilia A mice harboring clinically significant antihuman FVIII inhibitory antibody titers and resulted in high-level plasma FVIII activity and antibody titers that declined steadily throughout the course of the study, suggesting such a gene therapy strategy could be applicable even for patients with anti-FVIII antibodies [36].

Improved functional activity

Increased activation

The efficient activation of FVIII by thrombin is dependent on the presence of amino acid sequence Asp713–Arg740. In an effort to increase the sensitivity of FVIII to thrombin, Voorberg *et al*. replaced this region of FVIII with the amino acid sequence Ile51–Leu80 of the thrombin inhibitor heparin cofactor II, as this sequence was noted to be important for the inhibitory function of heparin cofactor II toward thrombin [37]. The hybrid molecule des-(868–1562)-FVIII–HCII was more readily activated and effectively reduced the clotting time of FVIII-deficient plasma.

Resistance to inactivation

Initial strategies to render FVIIIa resistant to inactivation targeted the FVIII residues that are the targets for aPC and FXa. However, single mutations at either Arg336 or Arg562 in FVIII were insufficient for complete resistance to aPC-mediated inactivation, whereas the double mutant Arg336Ile/Arg562Lys proved to be resistant [38]. These mutants do not exhibit higher specific activity and are still limited by A2 subunit dissociation *in vitro*.

In order to address this limitation, an inactivation-resistant FVIII (IR8) was genetically engineered which is not susceptible to dissociation of the A2 domain subunit and proteolytic

inactivation by aPC and therefore has a prolonged cofactor activity [39]. In designing IR8, B-domain residues 794–1689 were deleted and Arg740 was replaced by alanine, eliminating the thrombin-cleavage sites at Arg740 and Arg1689. As a result, FVIII activation by thrombin occurs via a single cleavage after Arg372. This leads to generation of a FVIIIa dimer that retains the A2 domain covalently attached to the light chain, thus preventing its spontaneous dissociation. Additionally, missense mutations at aPC inactivation cleavage sites provided resistance to further proteolysis of FVIIIa. The specific activity of IR8 proved to be fivefold higher than that of wild-type FVIII, and 38% of peak activity was retained even after 4 h *in vitro*, whereas wild-type FVIII, under similar conditions, was inactivated by thrombin after 10 min.

Gale and colleagues hypothesized that a disulfide bond engineered between the A2 and A3 domains could also stabilize FVIIIa by preventing A2 subunit dissociation following thrombin activation. They used a three-dimensional homology model of FVIII A domains [40] to identify residues 664 and 1826 as a potential disulfide bond pair. A BDD-FVIII mutant containing Cys664 and Cys1826 (C664–C1826) exhibited normal procoagulant specific activity [41]. However, following thrombin activation of C664–C1826, the mutant lost only 20% of its peak activity after 40 min at 22°C as measured by one-stage clotting assay. During this same incubation time, wild-type FVIIIa had lost more than 99% of its activity.

Both of the above strategies may significantly alter the folding and final tertiary structure of the FVIII molecule, raising concerns for potential immunogenicity. Wakabayashi and Fay have identified key residues that contribute to the A2 domain-dependent structural stability of FVIII [42]. This led to targeted bioengineering strategies to enhance the stability of FVIIIa. Charged residues Glu272, Asp519, Glu665, and Glu1984 are buried at the A2/A1 or A2/A3 domain interfaces. Replacement of these residues with either Ala or Val within a BDD-FVIII construct resulted in FVIII variants with wild-type-like activity but enhanced stability of FVIIIa [43]. Such variants resulted in enhanced thrombin generation *in vitro*. Further, selected combinations of mutations at these sites directed at reducing the charge and/or increasing the hydrophobicity at the A2/A1 and A2/A3 domain interfaces yielded factor VIII variants with improved FVIIIa stability. Such conservatively altered bioengineered FVIII variants could enhance the effective thrombin generation *in vivo* if incorporated into gene transfer strategies and may correct hemostasis *in vivo* at lower expression levels of protein.

Improved plasma half-life

Insights into the mechanisms of FVIII catabolism have provided direction for bioengineering strategies to prolong the half-life of FVIII in the circulation by generating FVIII mutants with disrupted interactions with its clearance receptors. FVIII catabolism is mediated by low-density lipoprotein receptor-related protein (LRP) [44], a hepatic clearance receptor with a broad ligand specificity that includes a number of proteins involved in blood coagulation and fibrinolysis. LRP-mediated clearance of FVIII from its complex with von Willebrand factor (VWF) is also facilitated by cell-surface heparan sulfate proteoglycans (HSPGs), one of the major glycoprotein components of the extracellular matrix [45]. Simultaneous blocking of both of these receptors led to a more significant prolongation of FVIII half-life in mice (5.5-fold) than the blocking of LRP alone (3.3-fold). The region of FVIII involved in binding to HSPGs was localized within the A2 domain residues 558–565 [45]. The current model for FVIII catabolism implicates the initial binding of FVIII–VWF complex to HSPGs, concentrating the complex on the cell surface and presenting it to LRP, followed by LRP-mediated catabolism of FVIII.

Study of a three-dimensional model of FVIII [46] revealed that the LRP binding site (residues 484–509) within the A2 domain contains six positively charged residues, three Lys at positions 493, 496, and 499 and three Arg at positions 484, 489, and 490, which are tightly clustered and form a prominently exposed cationic patch on the A2 surface. Two Lys residues at positions 556 and 570 and Arg571 are exposed within the HSPGs-binding site (residues 558–565) or in immediate proximity to it, and outside residues Lys380, Lys523, Lys659, and Lys661 are spatially close to the 558–565 region. These basic residues, which are potentially responsible for FVIII interactions with LRP and HSPGs, represent prospective targets for site-directed mutagenesis. Reduced affinity could be achieved by substitution of these residues either by neutral amino acids such as Ala or Ser or, alternatively, by oppositely charged residues (Glu), which may result in a more pronounced effect because of repulsion between the mutated residue and the corresponding negatively charged residues of LRP and HSPGs. This type of mutagenic approach has been previously shown for other LRP ligands [47,48] and for ligands of HSPGs [49]. In order to achieve maximal prolongation of FVIII half-life in the circulation, it may be necessary to combine mutations reducing FVIII interactions with both LRP and HSPGs. Suppression of FVIII interaction with its catabolic receptors LRP and HSPGs can potentially prolong FVIII lifetime in circulation and lead to higher plasma FVIII levels following gene transfer strategies for hemophilia A.

Factor IX with improved functional properties (Table 37.2)

Factor IX is significantly smaller than FVIII at only 415 amino acids and is secreted as a single-chain molecule. It also undergoes complex post-translational modifications, many of which are critical to its biochemical and pharmacokinetic properties [50]. Among all of these post-translational modifications, 12 glutamic acid residues near the amino-terminus of FIX are γ-carboxylated by the hepatic microsomal enzyme, vitamin K-dependent γ-glutamyl carboxylase. This is required for the

Table 37.2

Targeted functional improvement	Bioengineering strategy	References
Improved biosynthesis and secretion		
Increased mRNA expression	ATG triplets as translation initiation	55
	Truncated FIX intron 1	56, 57
Reduced collagen IV binding	Lys5Ala, Val10Lys	59
Improved functional activity		
Increaased specific activity	Arg338Ala	60
	FIX/FX hybrids	61

interaction of FIX with phospholipid surfaces and for optimal FIX activity. In addition, an amino-terminus propeptide sequence serves as a recognition signal for γ-glutamyl carboxylase and following γ-carboxylation is cleaved off by a Golgi apparatus serine protease known as paired basic amino acid cleaving enzyme (PACE/Furin) [51]. Additional complex post-translational modifications include β-hydroxylation, sulfation, phosphorylation and processing of the N-terminus [52,53]. The structure of recombinant FIX (rFIX) is similar to that of plasma-derived FIX, however, post-translational modifications of rFIX are similar but not identical to those of plasma-derived FIX [54]. Insights from the study of FIX biosynthesis, structure, and function have also identified potential targets for bioengineering strategies to enhance its functional properties.

Increased mRNA production

rFIX production level can be increased eightfold by including three ATG triplets as a translation initiation signal [55]. Similar to the observations with FVIII variants, introducing a truncated form of FIX intron 1 into the wild-type FIX cDNA strongly enhanced accumulation of FIX mRNA in the nucleus, most likely because of better protection of such transcripts from random degradation, and in turn led to an 8- to 15-fold increase in rFIX production both *in vitro* and *in vivo* [56–58].

Reduced binding to collagen IV

Phase I clinical trials have demonstrated that parenteral administration of AAV.2-mediated FIX gene transfer vectors into skeletal muscle or liver in humans is safe and well tolerated but therapeutic FIX levels have not yet been realized with current vector dose. Current strategies have been directed at vectorologic issues that contribute to host immune reactions. However, it is also hypothesized that FIX access to the plasma is limited by its expression in a tissue that is rich in collagen IV, such as the skeletal muscle. Schuettrumpf *et al*. proposed that mutants of FIX with defective binding to collagen IV (variants Lys5Ala and Val10Lys, designated K5A and V10K) may result in higher circulating FIX following AAV-mediated gene transfer to the skeletal muscle [59]. Mice injected intramuscularly with AAV encoding a bioengineered variant of FIX (FIX-V10K + K5A) had FIX levels increased fivefold compared with FIX-WT without compromise of FIX function or an immunologic response to the transgene. Staining for FIX in muscle revealed that FIX located in the extracellular space was substantially reduced using these FIX variants. These results suggest that the use of FIX variants can improve the efficacy of gene therapy for hemophilia B by increasing circulating levels of the protein without increasing the vector dose.

Increased specific activity

The variant FIX-R338A is associated in *in vitro* studies with threefold higher specific activity compared with FIX-WT [60]. This variant has also been investigated as an alternative approach for both liver or muscle-mediated gene transfer [59]. Similar to the experiments described above, mice injected intramuscularly with AAV FIX-R338A exhibited a threefold higher specific activity compared with FIX-WT. When FIX levels >500 ng/ml were obtained, a significant reduction in FIX specific activity was observed for both transgenes suggesting some limitations in post-translational modifications at higher expression levels. However, following liver-directed gene transfer of AAV.FIX-R338A, a sevenfold higher specific activity was observed compared with FIX-WT and a full correction of the aPTT was consistently obtained in hemophilia B knockout mice. In addition, no antibody formation was detected for this mutant when expressed in mice tolerized to human FIX-WT, again suggesting no increase in immunogenicity with this variant.

Hopfner *et al*. have utilized insights from the extensive structural and functional homology of FIXa and FXa to attempt to increase the catalytic potency of FIXa [61]. Using site-directed mutagenesis they swapped residues or loops between FIXa and FXa within recombinant variants that had deleted the Gla and EGF1 domains facilitating their expression in *Escherichia coli*. Specifically, they exchanged residues Glu219, Ile213, and the residues around Tyr99 and Lys148. When six residues adjacent to Tyr99 in FIX were replaced with the four equivalent residues from FX, they observed a 20-fold increase in the catalytic efficiency. Replacing residue Ile213 with Val produced a moderate increase whereas replacing Glu219 with Gly showed no increase in catalytic efficiency. Changing three residues adjacent to Lys148 within FIX to the equivalent residues from FX exhibited a similar effect as the Glu219Gly substitution. However, when all four mutations described above were combined there was an additive effect and the resultant quadruple mutant exhibited a catalytic efficiency 130-fold that of the wild-type FIX recombinant variant tested.

Thus, the use of FIX variants with higher specific activity could also improve the efficacy of gene therapy for hemophilia B by increasing the hemostatic efficacy of the expressed FIX without a need for increasing the vector dose.

Future directions

Advances in gene therapy vectorology and an increased understanding of the host immunologic responses will contribute to improved gene transfer strategies in the future. Incorporating bioengineered variants of FVIII or FIX, though an exciting prospect toward improving the therapeutic application of such technology, is not without some significant remaining challenges. There remains the potential for such bioengineered variants to have increased immunogenicity. The available small animal models have a propensity to inhibitor formation against human forms of FVIII and FIX. There remains a need for the development of animal models that are tolerant to human FVIII and FIX to then test bioengineered variants for increased immunogenicity. Porcine–human hybrids bioengineered for reduced immunogenicity [62] may also be able to address this concern. Secondly, hemophilia has been an attractive target for gene therapy studies as even low levels of FVIII or FIX expression would likely have therapeutic benefit. However, bioengineered variants with increased secretion efficiency, potency or reduced clearance, that achieve significant plasma levels may increase the thrombosis risk *in vivo*. Preclinical studies within small animal models will need to analyze the thrombogenicity of these variants over a wide range of plasma levels. Finally, as the functional properties of these bioengineered variants are altered, the standard activity and antigen assays that have been used as surrogates to guide therapy may no longer be useful. Novel bioassays may need to be developed or there may be renewed interest in some alternative global coagulation assays such as rotational thromboelastograms or thrombin generation assays.

References

1 Malhotra JD, Kaufman RJ. The endoplasmic reticulum and the unfolded protein response. *Semin Cell Dev Biol* 2007; **18**: 716–31.

2 Roth DA, Tawa NE, Jr., O'Brien JM, Treco DA, Selden RF. Nonviral transfer of the gene encoding coagulation factor VIII in patients with severe hemophilia A. *N Engl J Med* 2001; **344**: 1735–42.

3 Toole JJ, Pittman DD, Orr EC, Murtha P, Wasley LC, Kaufman RJ. A large region (approximately equal to 95 kDa) of human factor VIII is dispensable for *in vitro* procoagulant activity. *Proc Natl Acad Sci USA* 1986; **83**: 5939–42.

4 Pittman DD, Alderman EM, Tomkinson KN, Wang JH, Giles AR, Kaufman RJ. Biochemical, immunological, and *in vivo* functional characterization of B-domain-deleted factor VIII. *Blood* 1993; **81**: 2925–35.

5 Sandberg H, Almstedt A, Brandt J, *et al*. Structural and functional characteristics of the B-domain-deleted recombinant factor VIII protein, r-VIII SQ. *Thromb Haemost* 2001; **85**: 93–100.

6 Lusher JM, Lee CA, Kessler CM, Bedrosian CL. The safety and efficacy of B-domain deleted recombinant factor VIII concentrate in patients with severe haemophilia A. *Haemophilia* 2003; **9**: 38–49.

7 Courter SG, Bedrosian CL. Clinical evaluation of B-domain deleted recombinant factor VIII in previously untreated patients. *Semin Hematol* 2001; **38**(Suppl. 4): 44–59.

8 Mannucci PM. Hemophilia: treatment options in the twenty-first century. *J Thromb Haemost* 2003; **1**: 1349–55.

9 Kelley K, Verma I, Pierce GF. Gene therapy: reality or myth for the global bleeding disorders community? *Haemophilia* 2002; **8**: 261–7.

10 Plantier JL, Rodriguez MH, Enjolras N, Attali O, Negrier C. A factor VIII minigene comprising the truncated intron I of factor IX highly improves the *in vitro* production of factor VIII. *Thromb Haemost* 2001; **86**: 596–603.

11 Hoeben RC, Fallaux FJ, Cramer SJ, *et al*. Expression of the blood-clotting factor-VIII cDNA is repressed by a transcriptional silencer located in its coding region. *Blood* 1995; **85**: 2447–54.

12 Miao CH, Ye X, Thompson AR. High-level factor VIII gene expression *in vivo* achieved by nonviral liver-specific gene therapy vectors. *Hum Gene Ther* 2003; **14**: 1297–305.

13 Dorner AJ, Wasley LC, Kaufman RJ. Increased synthesis of secreted proteins induces expression of glucose-regulated proteins in butyrate-treated Chinese hamster ovary cells. *J Biol Chem* 1989; **264**: 20602–7.

14 Dorner AJ, Wasley LC, Kaufman RJ. Overexpression of GRP78 mitigates stress induction of glucose regulated proteins and blocks secretion of selective proteins in Chinese hamster ovary cells. *Embo J* 1992; **11**: 1563–71.

15 Dorner AJ, Wasley LC, Kaufman RJ. Protein dissociation from GRP78 and secretion are blocked by depletion of cellular ATP levels. *Proc Natl Acad Sci USA* 1990; **87**: 7429–32.

16 Tagliavacca L, Wang Q, Kaufman RJ. ATP-dependent dissociation of non-disulfide-linked aggregates of coagulation factor VIII is a rate-limiting step for secretion. *Biochemistry* 2000; **39**: 1973–81.

17 Marquette KA, Pittman DD, Kaufman RJ. A 110-amino acid region within the A1-domain of coagulation factor VIII inhibits secretion from mammalian cells. *J Biol Chem* 1995; **270**: 10297–303.

18 Swaroop M, Moussalli M, Pipe SW, Kaufman RJ. Mutagenesis of a potential immunoglobulin-binding protein-binding site enhances secretion of coagulation factor VIII. *J Biol Chem* 1997; **272**: 24121–4.

19 Pittman DD, Marquette KA, Kaufman RJ. Role of the B domain for factor VIII and factor V expression and function. *Blood* 1994; **84**: 4214–25.

20 Oeri J, Matter M, Isenschmid H, Hauser F, Koller F. Angeborener mangel an faktor V (parahaemophilie) verbunden mit echter haemophilie A bein zwei brudern. *Med Probl Paediatr* 1954; **1**: 575.

21 Herrmann JM, Malkus P, Schekman R. Out of the ER—outfitters, escorts and guides. *Trends Cell Biol* 1999; **9**: 5–7.

22 Spreafico M, Peyvandi F. Combined FV and FVIII deficiency. *Haemophilia* 2008; **14**: 1201–8.

23 Moussalli M, Pipe SW, Hauri HP, Nichols WC, Ginsburg D, Kaufman RJ. Mannose-dependent endoplasmic reticulum (ER)-Golgi intermediate compartment-53-mediated ER to Golgi trafficking of coagulation factors V and VIII. *J Biol Chem* 1999; **274**: 32539–42.

24 Zhang B, Cunningham MA, Nichols WC, *et al*. Bleeding due to disruption of a cargo-specific ER-to-Golgi transport complex. *Nat Genet* 2003; **34**: 220–5.

25 Kawasaki N, Ichikawa Y, Matsuo I, *et al*. The sugar-binding ability of ERGIC-53 is enhanced by its interaction with MCFD2. *Blood* 2008; **111**: 1972–9.

26 Guy JE, Wigren E, Svard M, Hard T, Lindqvist Y. New insights into multiple coagulation factor deficiency from the solution structure of human MCFD2. *J Mol Biol* 2008; **381**: 941–55.

27 Zhang B, Kaufman RJ, Ginsburg D. LMAN1 and MCFD2 form a cargo receptor complex and interact with coagulation factor VIII in the early secretory pathway. *J Biol Chem* 2005; **280**: 25881–6.

28 Miao HZ, Sirachainan N, Palmer L, *et al*. Bioengineering of coagulation factor VIII for improved secretion. *Blood* 2004; **103**: 3412–19.
29 Cerullo V, Seiler MP, Mane V, *et al*. Correction of murine hemophilia A and immunological differences of factor VIII variants delivered by helper-dependent adenoviral vectors. *Mol Ther* 2007; **15**: 2080–7.
30 Malhotra JD, Miao H, Zhang K, *et al*. Antioxidants reduce endoplasmic reticulum stress and improve protein secretion. *Proc Natl Acad Sci USA* 2008; **105**: 18525–30.
31 High KA. Update on progress and hurdles in novel genetic therapies for hemophilia. *Am Soc Hematol Educ Program* 2007: 466–72.
32 Jirovska D, Ye P, Pipe SW, Miao CH. Reduction of inhibitory anti-FVIII antibody titer by using a B domain variant FVIII/N6 cDNA for nonviral gene therapy in hemophilia A mice. *ASH Annual Meeting Abstracts* 2008; **112**: 3537.
33 Doering CB, Healey JF, Parker ET, Barrow RT, Lollar P. High level expression of recombinant porcine coagulation factor VIII. *J Biol Chem* 2002; **277**: 38345–9.
34 Doering CB, Healey JF, Parker ET, Barrow RT, Lollar P. Identification of porcine coagulation factor VIII domains responsible for high level expression via enhanced secretion. *J Biol Chem* 2004; **279**: 6546–52.
35 Ide LM, Gangadharan B, Chiang KY, Doering CB, Spencer HT. Hematopoietic stem-cell gene therapy of hemophilia A incorporating a porcine factor VIII transgene and nonmyeloablative conditioning regimens. *Blood* 2007; **110**: 2855–63.
36 Doering CB, Gangadharan B, Dukart HZ, Spencer HT. Hematopoietic stem cells encoding porcine factor VIII induce procoagulant activity in hemophilia A mice with pre-existing factor VIII immunity. *Mol Ther* 2007; **15**: 1093–9.
37 Voorberg J, van Stempvoort G, Bos JM, Mertens K, van Mourik JA, Donath MJ. Enhanced thrombin sensitivity of a factor VIII-heparin cofactor II hybrid. *J Biol Chem* 1996; **271**: 20985–8.
38 Amano K, Michnick DA, Moussalli M, Kaufman RJ. Mutation at either Arg336 or Arg562 in factor VIII is insufficient for complete resistance to activated protein C (APC)-mediated inactivation: implications for the APC resistance test. *Thromb Haemost* 1998; **79**: 557–63.
39 Pipe SW, Kaufman RJ. Characterization of a genetically engineered inactivation-resistant coagulation factor VIIIa. *Proc Natl Acad Sci USA* 1997; **94**: 11851–6.
40 Pemberton S, Lindley P, Zaitsev V, Card G, Tuddenham EG, Kemball-Cook G. A molecular model for the triplicated A domains of human factor VIII based on the crystal structure of human ceruloplasmin. *Blood* 1997; **89**: 2413–21.
41 Gale AJ, Pellequer JL, Griffin JH. A novel engineered interdomain disulfide bond stabilizes human blood coagulation factor VIIIa. *J Thromb Haemost* 2003; **1**(Suppl. 1): OC094.
42 Wakabayashi H, Fay PJ. Identification of residues contributing to A2 domain-dependent structural stability in factor VIII and factor VIIIa. *J Biol Chem* 2008; **283**: 11645–51.
43 Wakabayashi H, Varfaj F, Deangelis J, Fay PJ. Generation of enhanced stability factor VIII variants by replacement of charged residues at the A2 domain interface. *Blood* 2008; **112**: 2761–9.
44 Schwarz HP, Lenting PJ, Binder B, *et al*. Involvement of low-density lipoprotein receptor-related protein (LRP) in the clearance of factor VIII in von Willebrand factor-deficient mice. *Blood* 2000; **95**: 1703–8.
45 Sarafanov AG, Ananyeva NM, Shima M, Saenko EL. Cell surface heparan sulfate proteoglycans participate in factor VIII catabolism mediated by low density lipoprotein receptor-related protein. *J Biol Chem* 2001; **276**: 11970–9.
46 Stoilova-McPhie S, Villoutreix BO, Mertens K, Kemball-Cook G, Holzenburg A. 3-Dimensional structure of membrane-bound coagulation factor VIII: modeling of the factor VIII heterodimer within a 3-dimensional density map derived by electron crystallography. *Blood* 2002; **99**: 1215–23.
47 Rodenburg KW, Kjoller L, Petersen HH, Andreasen PA. Binding of urokinase-type plasminogen activator-plasminogen activator inhibitor-1 complex to the endocytosis receptors alpha2-macroglobulin receptor/low-density lipoprotein receptor-related protein and very-low-density lipoprotein receptor involves basic residues in the inhibitor. *Biochem J* 1998; **329**: 55–63.
48 Howard GC, Yamaguchi Y, Misra UK, *et al*. Selective mutations in cloned and expressed alpha-macroglobulin receptor binding fragment alter binding to either the alpha2-macroglobulin signaling receptor or the low density lipoprotein receptor-related protein/alpha2-macroglobulin receptor. *J Biol Chem* 1996; **271**: 14105–11.
49 Knauer MF, Kridel SJ, Hawley SB, Knauer DJ. The efficient catabolism of thrombin-protease nexin 1 complexes is a synergistic mechanism that requires both the LDL receptor-related protein and cell surface heparins. *J Biol Chem* 1997; **272**: 29039–45.
50 White GC, 2nd, Beebe A, Nielsen B. Recombinant factor IX. *Thromb Haemost* 1997; **78**: 261–5.
51 Wasley LC, Rehemtulla A, Bristol JA, Kaufman RJ. PACE/furin can process the vitamin K-dependent pro-factor IX precursor within the secretory pathway. *J Biol Chem* 1993; **268**: 8458–65.
52 Suttie JW. Synthesis of vitamin K-dependent proteins. *FASEB J* 1993; **7**: 445–52.
53 Kurachi K, Davie EW. Isolation and characterization of a cDNA coding for human factor IX. *Proc Natl Acad Sci USA* 1982; **79**: 6461–4.
54 Bond M, Jankowski M, Patel H, *et al*. Biochemical characterization of recombinant factor IX. *Semin Hematol* 1998; **35**(Suppl. 2): 11–17.
55 Enjolras N, Rodriguez MH, Plantier JL, Maurice M, Attali O, Negrier C. The three in-frame ATG, clustered in the translation initiation sequence of human factor IX gene, are required for an optimal protein production. *Thromb Haemost* 1999; **82**: 1264–9.
56 Kurachi S, Hitomi Y, Furukawa M, Kurachi K. Role of intron I in expression of the human factor IX gene. *J Biol Chem* 1995; **270**: 5276–81.
57 Zheng B, Qiu XY, Tan M, *et al*. Increment of hFIX expression with endogenous intron 1 *in vitro*. *Cell Res* 1997; **7**: 21–9.
58 Rodriguez MH, Enjolras N, Plantier JL, *et al*. Expression of coagulation factor IX in a haematopoietic cell line. *Thromb Haemost* 2002; **87**: 366–73.
59 Schuettrumpf J, Herzog RW, Kaufhold A, Stafford DW, High KA, Arruda VR. Improving efficacy of gene therapy of hemophilia B by the use of mutant FIX variants. *J Thromb Haemost* 2003; **1**(Suppl. 1): OC044.
60 Chang J, Jin J, Lollar P, *et al*. Changing residue 338 in human factor IX from arginine to alanine causes an increase in catalytic activity. *J Biol Chem* 1998; **273**: 12089–94.
61 Hopfner KP, Brandstetter H, Karcher A, *et al*. Converting blood coagulation factor IXa into factor Xa: dramatic increase in amidolytic activity identifies important active site determinants. *Embo J* 1997; **16**: 6626–35.
62 Barrow RT, Healey JF, Gailani D, Scandella D, Lollar P. Reduction of the antigenicity of factor VIII toward complex inhibitory antibody plasmas using multiply-substituted hybrid human/porcine factor VIII molecules. *Blood* 2000; **95**: 564–8.

38 Laboratory assays in hemophilia

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Introduction

During the coagulation process, factor VIII (FVIII) and FIX provide their natural support of the conversion of FX into FXa, thereby creating the biochemical basis for generation of thrombin, which catalyzes the production of a fibrin clot. In hemophilia, the lack of FVIII or FIX dramatically reduces the capacity of this intrinsic Xase reaction and, as a consequence, there is a significantly delayed formation of insufficient amounts of fragile fibrin strands, which are unable to provide robust hemostasis.

In a hemophilic patient who receives infusions of factor concentrate sufficient to restore incrementally the factor level to 100% of normal, hemostasis should be similar to that in a nonhemophilic individual. An unresolved issue in the management of hemophilia is lack of evidence for a critical factor level that determines the arrest of bleeding and maintains hemostasis. Instead, weight-based dosage guidelines for various types of bleed and surgery are quite commonplace in hemophilia management today, despite the fact that a heterogeneic response to treatment among patients is well recognized. The obvious lack of evidence from larger randomized controlled clinical trials creates a huge demand for laboratory monitoring of treatment in patients.

The assays commonly used in hemophilia laboratory services for diagnosis, classification, and therapy monitoring will be addressed and discussed here, and some general comments on their utility will be given. Since manufacturers of coagulation instruments and reagents provide consumers with complete protocols for recording of coagulation factors, individual assay protocols will not be considered.

The fundamentals of biometry of FVIII and FIX have previously been reported in detail in an excellent monograph, dated 1984 [1–4].

About factor VIII and IX

The FVIII molecule is undoubtedly one of the most unstable of the frequently recorded coagulation factors. FVIII displays a great ability to absorb onto foreign surfaces, and it degrades quickly when exposed to ambient temperature, if the pH value falls outside a rather narrow range, and if the FVIII molecule is exposed to natural proteolytic plasma enzymes that irreversibly inactivate FVIII. Contributing to accuracy problems, samples may give different results with different reagents and instruments.

In most of these respects, the FIX molecule is less sensitive to storage and assay conditions.

Laboratory work-up for the diagnosis of hemophilia

Table 38.1 lists analytic procedures commonly used to establish the diagnosis of hemophilia, simultaneously ruling out related bleeding disorders.

The activated partial thromboplastin time

In most instances, a prolonged activated partial thromboplastin time (aPTT) detected in an otherwise healthy male suffering an increased tendency to bleeding is the hallmark of a deficiency state of a coagulation factor belonging to the contact pathway of coagulation, raising the suspicion of hemophilia. A multitude of different aPTT reagents are available on the market. Variance in response is related to the nature of the activating substance as well as to the type and composition of phospholipids. In most instances, protocols are provided by the manufacturer for their use with various kinds of coagulation instruments. When selecting a particular aPTT reagent for routine use in hemophilia management, there are two main points to consider: (i) the sensitivity of the reagent to detect all severity classes of hemophilia (severe, moderate, and mild) and (ii) the suitability of the reagent in assays of single coagulation factor determination. In the daily routine, most laboratories find it most practical to use the same aPTT reagent for screening and activity determination of factors in order to avoid performance errors.

Further, the content and composition of the aPTT phospholipids reagent may be an important determinant for accurate assessment of recombinant FVIII in (postinfusion) hemophilic plasma samples [5]. The assay procedure should follow the guidelines specified by the manufacturer, in particular the proposed time for preactivation prior to addition of calcium.

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Table 38.1 Laboratory procedures commonly used in the diagnosis of hemophilia.

Platelet count	Platelet aggregation
	Agonists: collagen, ADP, ristocetin
aPTT	Activated partial thromboplastin time
PT	Prothrombin time
Factor VIII:C	Factor VIII procoagulant function (one-stage and/or chromogenic substrate method)
Factor VIII:Ag	Factor VIII antigenic determination
VWF:RCO	von Willebrand factor ristocetin cofactor
VWF:AG	von Willebrand factor antigen
VWF:FVIIIB	von Willebrand factor/FVIII-binding capacity
Fibrinogen	Functional assay for fibrinogen
Factor XIII	Enzymatic factor XIII assay

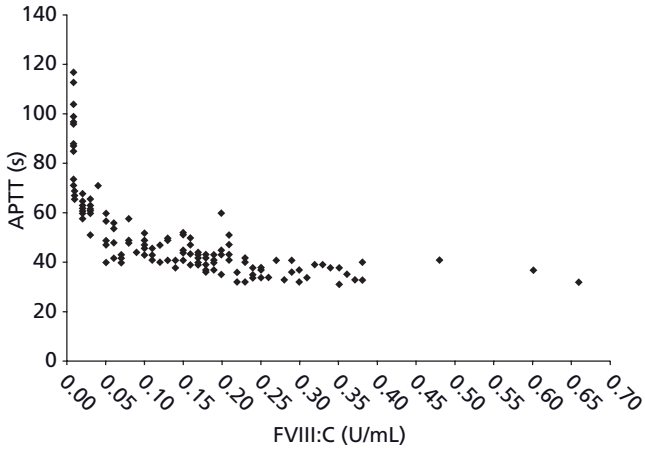

Figure 38.1 The relationship between factor VIII:C levels and aPTT in 140 plasma samples from patients with hemophilia A and five from patients with a reduced level of FVIII:C. The aPTT reagent was Platelin Excel (Organon Teknika, Turnhout, Belgium) and the aPTT and FVIII:C assays were carried out by the one-stage technique.

Keeping in mind the successes achieved with the widely adopted international normalized ratio (INR) system used for control of vitamin K antagonist treatment, several proposals have been made for standardized principles for estimation of the aPTT. However, the multitude of phospholipids and activators of various aPTT reagents have proved major obstacles in standardization, and as of today no recommended standard procedure exists.

Which kind of information does the crude aPTT really provide in practical use in hemophilia? Figure 38.1 illustrates graphically a large number of raw aPTT results determined in hemophilia A patients and a few people with a reduced FVIII plotted against the corresponding one-stage FVIII:C value recorded in the same sample. As shown, and as is well known to many physicians, severely affected hemophilia patients display a huge interpatient variation in the aPTT time. In moderate and mild hemophilia A, there is a quite useful correlation with FVIII:C levels from around 0.02 U/mL, but the aPTT itself is a rather poor predictor of the correct diagnostic class or the outcome of the FVIII:C level recording.

Factor VIII activity (FVIII:C) measured by the one-stage technique

The assay for recording of FVIII:C in plasma by the one-stage technique represents an extension of the aPTT assay. It relies on the assumption that the time of clotting is a function of the level of FVIII:C in a reaction system where FVIII is the only variable, whereas all other plasma coagulation factors are constant and normal. The test base is severe hemophilia A plasma (SHP) and the primary standard should be the current International Standard for Plasma FVIII. Secondary standards (in-house plasma pool or commercial standard) are used in the daily routine assays following local recalibration against the International Standard or other well-documented standard material. Calibration is recommended when analytic conditions change (i.e., shift of batch of aPTT reagent, change of secondary standard, shift of pool of SHP, etc.).

Deficiency plasmas

In the routine laboratory, severe hemophilia plasma (SHP) is best obtained from a well-characterized patient with severe hemophilia A (residual FVIII:C level <0.01 IU/mL, FVIII:Ag <0.01 IU/mL, and no detectable inhibitors) who has not received FVIII concentrate for 2 weeks or more. Owing to frequent treatment or prophylaxis, such material may be difficult to obtain today, and concern about laboratory operator safety often limits the use of plasma derived from patients. Commercial FVIII-deficient plasmas are usually produced by immunodepletion techniques or other principles, which may not completely remove FVIII molecules from the (healthy donor) raw plasma. The presence of tiny amounts of residual FVIII:C activity or FVIII:Ag may compromise the classification of the unknown hemophilia A patient, hamper the recording of postinfusion values of FVIII:C following administration of highly purified FVIII concentrates, and reduce the sensitivity of the assay for inhibitors against FVIII. Among commercial FVIII deficiency plasmas, some are also depleted in von Willebrand factor, which may increase the risk of accuracy problems in estimation of postdosage FVIII by the one-stage techniques following infusion of recombinant FVIII concentrates.

Calibration of the assay

Analytic variance is a major issue in FVIII:C recording. The automated instruments used today often have a memory function that saves calibration curves for various lengths of time. While such systems may save money and time for procedures recording several other components, they are not well chosen for FVIII measurements.

The calibration procedure is critical in recording of FVIII:C by the one-stage assay. A freshly prepared standard curve made up by several dilution steps of standard in SHP should be used in every series of FVIII:C measurements carried out. Double determinations are essential, and unknown sample material should preferably be tested at two or more different dilution steps to insure a parallel reaction versus standard.

Factor VIII:C measured by two-stage assays

In the past, the two-stage technique was often employed for its more accurate measurement of FVIII:C. This method is based on the activation of FX aided by activated FVIII and excess activated FIX, FVIIIa being the rate-limiting factor in the assay. In the second step, the function of FXa is recorded. A newer version of the two-stage assay, called the chromogenic substrate assay or peptide amidolytic assay, has replaced the traditional two-stage assay that was used in most clinical laboratories several years ago.

The principal reaction steps of the chromogenic assay are outlined in Figure 38.2.

The chromogenic assay methods record the enzymatic activity of activated FX (FXa), which is formed by the concerted action of FVIII (thrombin-) activated into FVIIIa and a preformed excess of FIXa in an environment containing phospholipids. Similar to the traditional two-stage assay, FVIIIa is the rate-limiting factor in the chromogenic reaction.

In certain respects, the critical requirements in the chromogenic assay are similar to those of the one-stage assay. A multiple dilution point standard curve should be used. If very low levels of FVIII:C are expected, a separate calibration curve should be adopted for recordings at the lower end of the dynamic measurement range (below 0.20 IU/mL FVIII:C). Manufacturers' recommended preactivation time (activation of FVIII to FVIIIa) should be followed strictly. The principles for standardization and calibration of the chromogenic assay are similar to those mentioned for the one-stage assay. A clear advantage of the chromogenic assay is improved precision; often the coefficient of variation (CV) is half of that obtained with the one-stage assay, as reported in several publications.

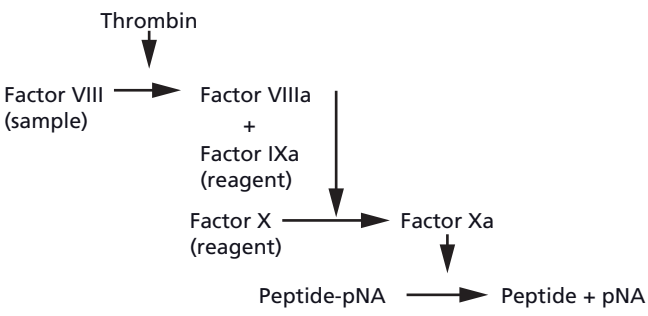

Figure 38.2 Reaction scheme steps in the chromogenic substrate method for measurement of factor VIII:C. The release of yellow free pNA molecules depends on the amount of FVIII in the sample.

Quality control systems in the hemostasis laboratory

In addition to the use of in-assay control procedures, which are mandatory in coagulometry, as in all other laboratory procedures, participation in national and international proficiency programs is very useful. Two major nonprofit organizations exist to serve laboratories on a multinational basis. In Europe, the ECAT Foundation (www.ecat.nl) annually distributes four sets of samples for proficiency testing of hemostasis components as well as a broad-based thrombophilia program. In North America, a collaborating sister organization, NASCOLA (www.nascola.org), offers a similar program. These quality assessment programs are highly valuable and of great benefit to participants.

Assay discrepancy

With some patient sample material, inconsistent results are found when comparing results of recordings of FVIII:C by the one-stage assay with those obtained with the chromogenic assay, or the two-stage clotting assay. This difference is often referred to using the term "assay discrepancy." The most likely explanation for the assay discrepancy phenomenon is differences in the way in which FVIII is activated with the two types of assays.

The FVIII activation during the course of the aPTT-based one-stage assay most likely depends on the endogenous formation of thrombin in the reaction mixture, while the chromogenic assay reagents contain exogenous thrombin that directly activates FVIII, and further thrombin formation is quenched through the use of a specific thrombin inhibitor.

Assay discrepancy has predominantly been observed in samples from certain subsets of mild hemophilia A patients, in high-purity FVIII concentrates, and in postinfusion samples from hemophilia patients undergoing treatment with FVIII concentrates purified by monoclonal antibody techniques and, in particular, recombinant FVIII concentrates.

Assay discrepancy in mild hemophilia

The assay discrepancy phenomenon in mild hemophilia was initially reported with the traditional two-stage assay for FVIII:C [6,7]. This phenomenon is now known to be quite prevalent [8]. Figure 38.3 illustrates the assay discrepancy phenomenon in mild hemophilia. Plasma samples from 69 nonrecently infused patients with mild hemophilia A from 43 families were recorded by the one-stage assay and a chromogenic assay. The figure is a differential plot in which the difference between the one-stage FVIII:C result and the corresponding chromogenic FVIII:C value is plotted. If results are concordant, data should be located close to the zero line of the plot. However, a majority of patients display a significant difference between the two. In one of our families, as well as in numerous families worldwide, the assay discrepancy phenomenon in

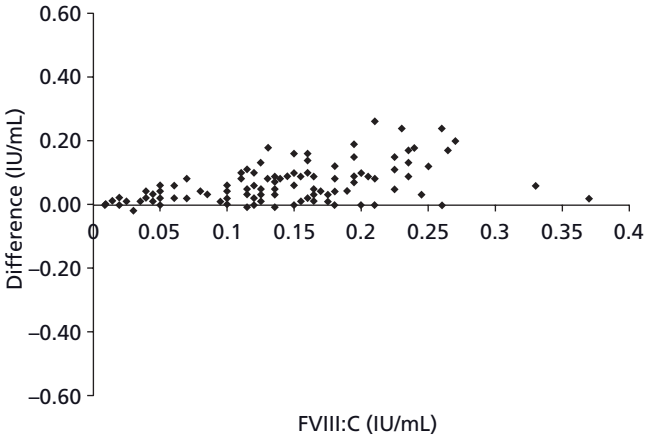

Figure 38.3 Differential plot of FVIII:C assay recorded by the one-stage method and by the chromogenic substrate method in 69 patients with moderate and mild hemophilia A from 43 Danish families. In each case, the chromogenic method result is subtracted from the one-stage result.

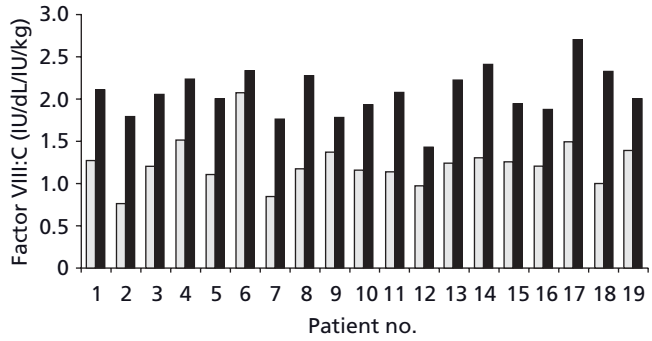

Figure 38.4 Assay discrepancy in plasma from each of 19 hemophilia A patients. The sample was collected 15 min after infusion of around 25 IU/kg of a B-domain-deleted recombinant FVIII product. Gray bars: FVIII:C incremental value determined by a one-stage FVIII:C assay; black bars: FVIII:C incremental value determined by a chromogenic substrate assay for FVIII:C. The expected incremental value is around 2.00.

mild hemophilia was found to be associated with missense mutations causing a premature disruption of the A2 domain when exposed to thrombin [9,10]. In these examples, the one-stage assay gave a higher FVIII:C activity compared with the chromogenic assay, but cases have also been identified with the opposite finding, and a normal chromogenic FVIII:C result [11,12].

Assay discrepancy in postinfusion samples following concentrate administration

In clinical practice, assay discrepancy has been reported in hemophilia A patients undergoing treatment with recombinant FVIII products. In general, the assay discrepancy reported is in the range of 10–30%, the one-stage assay persistently giving the lowest result [13,14]. Most markedly, discrepant results have been found with a B-domain-deleted (BDD) recombinant FVIII concentrate. An illustration of this is found in Figure 38.4, which shows a series of 19 patients with hemophilia in whom we routinely assessed the recovery of FVIII following infusion of a dose of around 25 IU/kg BDD recombinant FVIII. The response was calculated as the incremental rise in IU/dL divided by IU/kg b.w. (body weight) as measured by a one-stage assay as well as by a chromogenic substrate assay. The common standard was a plasma pool previously calibrated against the fourth international standard for FVIII in plasma. As shown, discrepant results were observed in all cases comparing the two types of assay, and the mean response was 1.27 IU/mL for the one-stage and 2.07 IU/mL for the chromogenic substrate assay. Overall, the mean ratio of one-stage FVIII:C/chromogenic FVIII:C was 0.61. Moreover, as can be seen from Figure 38.4, the assay discrepancy appears to vary substantially between patients. The assay discrepancy phenomenon with BDD recombinant FVIII can more or less be abolished using modified one-stage assays in which phospholipids are changed in composition or concentration [15,16].

When recording FVIII in postinfusion samples, the biologic effects of concentrate are similar to those of diluting concentrate in FVIII-deficient plasma *in vitro*, and the coagulometric response is expected to be similar to that obtained by adding concentrate to SHP *ex vivo*. Therefore, when recording the response to infusion of recombinant BDD FVIII, the International Society on Thrombosis and Hemostasis (ISTH) has recommended the use of the "like-versus-like" principle in which a concentrate standard of the same manufacture is used for calibration of the one-stage assay employed. A multilaboratory field study has demonstrated that the assay discrepancy phenomenon is abolished when the aforementioned strategy is adopted [17]. In contrast, since the chromogenic assay is not influenced by the type of concentrate infused when recorded against a plasma FVIII standard, the chromogenic analysis principle performs well with a plasma-based FVIII calibrator. For full-length recombinant FVIII concentrates, no recommendations have been endorsed on the use of concentrate-specific standard material at this time.

For accurate determination of FVIII in all kinds of biologic materials, it would be highly desirable to have a common assay that is unaffected by the purity of FVIII or FIX in concentrates and that is also unaffected by matrix effects. Such a system has recently been developed and tested for its suitability in recording of FVIII of varying purity and composition [18].

Factor IX:C measurements

In recording FIX:C, the fundamental principles of the assay system are the same as those governing the measurement of

FVIII:C: the use of the international standard for plasma FIX:C as the primary standard for calibration of the assay, the need for a well-controlled severely FIX:C-deficient plasma test base, and the use of controls.

Chromogenic peptide assay systems do exist for recording of FIX:C, but such assays have not been challenged in formal standardization procedures and assays of this kind are not widely used in clinical hemostasis laboratories.

Models for studying the entire process of coagulation

In daily laboratory practice, determination of hemophilia is based on the use of citrated plasma, and the initial signal of coagulation is utilized in all methods based on the global aPTT and prothrombin time (PT) assays, the endpoint of the analysis being determined by a change in light transmission or in viscoelastic mechanical resistance. When measuring FVIII and FIX activities using clotting assays, the same endpoint principles are utilized. For around half a century, researchers have speculated whether surveillance of the entire process of coagulation might provide more information on the pathology of coagulation in various hemorrhagic disorders. An early finding was that a considerable amount of thrombin activity is generated in plasma over a period of several minutes after the initial signs of fibrin formation [17] demonstrable in continuously collected subsamples from the reaction mixture. Another early method (the thromboelastograph) characterizes the course of coagulation by means of the mechanical elasticity changes occurring in whole blood during fibrin polymerization. Although these assays differ in one respect, one being based on diluted and defibrinated plasma with or without platelets and the other using undiluted whole blood, the general characteristics of the courses of thrombin generation and fibrin formation appear to be quite similar in healthy subjects. More detailed description on the continuous methods and the interpretation of their results is found in Chapter 40 (Benny Sørensen).

Determination of the antigens of factor VIII and factor IX

Estimation of the antigenic concentration of the coagulation factor lacking in patients' blood has several advantages, especially for phenotyping. A CRM^- (cross-reacting material negative) status (low or no recordable antigen) is most often associated with a severe bleeding condition and linkage to a genetic defect that may predict a high-risk of inhibitor formation, whereas a CRM^+ condition (excess of antigen over activity) is rarely found in patients with severe hemophilia but is often seen in milder cases.

For several years, immunoradiometric assays or enzyme-linked immunosorbent assays (ELISAs) have existed for quantitation of FVIII antigenic concentration, and antibodies derived from patients with inhibitors were most often used in such assays. Since ultrapure FVIII was difficult to obtain and immunization of animals often resulted in antibodies with cross-reactivity to other plasma proteins, only a few laboratories were capable of recording FVIII:Ag. Today, home-made as well as commercial ELISA systems are more widely available for quantitation of FVIII:Ag (see [19]).

FIX:Ag is quite easily recorded by ELISA-based methods, and commercial kits and home-made methods are in widespread use.

Inhibitors to factor VIII and factor IX

The Bethesda assay and the Nijmegen modified assay variants

The classic test system for determination of inhibitors against FVIII and FIX is the Bethesda assay, which was published in 1975. In principle, this recording system is based on the well-known principle that inhibitors inactivate the FVIII or FIX molecules of normal plasma. In practice, several different dilutions of patient's plasma are added to a normal plasma sample and incubated for 2 h at 37°C, at the end of which all samples are recorded for their residual content of FVIII:C or FIX:C. A positive result is noted when a sample causes a significant loss of FVIII:C or FIX:C of normal plasma at 50% of the content of the control sample. Dilutions and residual FVIII:C are plotted against each other and the inhibitor titer is obtained by linear regression. This system was unchanged for 20 years until a modified assay was published in which the natural instability of FVIII and the change of pH occurring during the rather lengthy incubation period were compensated for by the use of buffered normal plasma, and by including SHP in the control mixture [20,21]. Using this assay, a number of false-positive inhibitors could largely be excluded. Later, it was found that SHP could be replaced by a 4% albumin solution [22]. Since then, the Nijmegen modification of the Bethesda assay has been increasingly adopted in clinical laboratory work. Two major problems remain unresolved: the low accuracy of inhibitor assays in general and the lack of consensus in terms of the low detection limit of inhibitors by functional methods (most often referred to as 0.6 Bethesda units per mL). The accuracy problem is tightly linked with the inherent imprecision of the one-stage FVIII assay.

In order to overcome this problem to some extent, attempts have been made to record inhibitors using a chromogenic substrate assay for FVIII. No larger series of patient samples have as yet been published on the chromogenic method. In a smaller study, inhibitor titers were lower when measured with the chromogenic assay as compared with the one-stage assay result (S. Raut, National Institute for Biological Standards and Control, Potter's Bar, Hertfordshire, UK, personal communication).

Detecting inhibitors using immunologic methods

Among physicians it is generally well known that the Bethesda assay is insensitive to weakly reacting inhibitors, the presence of which may be observed only by a reduced *in vivo* recovery of infused concentrate or a shortened *in vivo* half-life of concentrate administered. Two principal methods of detecting such nonmeasurable inhibitors have been introduced. In some cases, patient antibodies may be detected by their capacity to immunoprecipitate FVIII. However, in a series of patients from Germany, the immunoprecipitation method did not detect inhibitors that were undetectable by the Bethesda assay [23].

Second, various ELISAs have been developed demonstrating increased sensitivity to weakly titered antibodies [24–26]. In interpreting the results of both of the aforementioned assays, noninhibitory antibodies against FVIII cannot be distinguished from inhibiting antibodies. Several investigators have shown that noninhibiting antibodies against FVIII are a quite frequent and harmless finding in healthy individuals. This means that detection of an antibody against FVIII using immunologic methods in hemophilia patients could raise suspicion of an inhibitor, but the specificity of the results is dubious and their predictive value questionable.

Conclusion

Although, today, sufficient amounts of concentrates with a high safety profile are available for the treatment of hemophilia, the hemostasis laboratory still has an important role to play in correctly determining the phenotype of patients with a hemorrhagic disorder and in ensuring optimal substitution treatment. There is an urgent need for appropriate point-of-care methods in hemophilia to help tailor individualized treatment protocols.

References

1 Over J. Methodology of the one-stage assay of factor VIII (VIII:C). *Scand J Haematol* 1984; **33**: 13–24.
2 Barrowcliffe T. Methodology of the two-stage assays of factor VIII. *Scand J Haematol* 1984; **33**: 25–38.
3 Barrowcliffe T. Comparisons of one-stage and two-stage assays of factor VIII:C. *Scand J Haematol* 1984; **33**: 39–54.
4 Curtis A. The statistical evaluation of factor VIII clotting assays. *Scand J Haematol* 1984; **33**: 55–70.
5 Mikaelsson M, Oswaldsson U, Sandberg H. Influence of phospholipids on the assessment of factor VIII activity. *Haemophilia* 1998; **4**: 646–50.
6 Parquet-Gernez A, Mazurier C, Goudemand M. Functional and immunological assays of FVIII in 133 haemophiliacs—characterization of a subgroup of patients with mild haemophilia A and discrepancy in 1- and 2-stage assays. *Thromb Haemost* 1988; **59**: 202–6.
7 Rudzki Z, Duncan EM, Casey GJ, *et al.* Mutations in a subgroup of patients with mild haemophilia A and discrepancy between the one-stage and two-stage factor VIII:C methods. *Br Haematol* 1996; **94**: 400–6.
8 Poulsen AL, Pedersen LH, Hvas AM, Poulsen LH, Thykjaer H, Ingerslev J. Assay discrepancy in mild haemophilia A: entire population study in a National Haemophilia Centre. *Haemophilia* 2009; **15**: 285–9.
9 Pipe SW, Eickhorst AN, McKinley SH, *et al.* Mild hemophilia A caused by increased rate of factor VIII A2 subunit dissociation: evidence for nonproteolytic inactivation of factor VIII *in vivo*. *Blood* 1999; **93**: 176–83.
10 Schwaab R, Oldenburg J, Kemball-Cook G, *et al.* Assay discrepancy in mild haemophilia A due to a factor VIII missense mutation (Asn694Ile) in a large Danish family. *Br J Haematol* 2000; **109**: 523–8.
11 Mumford AD, Laffan M, O'Donnell J, *et al.* A Tyr346 ® Cys substitution in the interdomain acidic region a1 of factor VIII in an individual with factor VIII:C assay discrepancy. *Br J Haematol* 2002; **118**: 589–94.
12 Bowyer AE, Kitchen S, Makris M. The chromogenic FVIII:C assay is a suitable alternative to the two-stage assay in detection of mild haemophilia A patients with one-stage, two-stage discrepancy. *J Thromb Haemost* 2007; 5: P-S-122.
13 Lee CA, Owens D, Bray G, *et al.* Pharmacokinetics of recombinant factor VIII (Recombinate) using one-stage clotting and chromogenic factor VIII assay. *Thromb Haemost* 1999; **82**: 1644–7.
14 Morfini M, Cinotti S, Belatreccia A, *et al.* A multicenter pharmacokinetic study of the B-domain deleted recombinant factor VIII concentrate using different assays and standards. *J Thromb Haemost* 2003; **1**: 2283–9.
15 Mikaelsson M, Oswaldsson U, Sandberg H. Influence of phospholipids on the assessment of factor VIII activity. *Haemophilia* 1998; **4**: 646–50.
16 Caron C, Dautzenberg M-D, Delahousse B, *et al.* A blinded *in vitro* study with Refacto mock plasma samples: similar F VIII results between the chromogenic assay and a one-stage assay using higher cephalin dilution. *Haemophilia* 2002; **8**: 639–43.
17 Ingerslev J, Jankowski MJ, Weston SB, *et al.* Collaborative field study on the utility of a BDD Factor VIII concentrate standard in the estimation of BDD Factor VIII:C activity in hemophilic plasma using one-stage clotting assays. *J Thromb Haemost* 2004; **2**: 623–8.
18 Eich S, Kusch M, Grundmann C, *et al.* Factor VIII determination in patients' plasma and concentrates: a novel test equally suited for both matrices. *Blood Coagul Fibrinol* 2003; **14**: 347–53.
19 Girma J-P, Fressinaud E, Houllier A, *et al.* Assay of factor VIII antigen (VIII:CAg) in 294 haemophilia A patients by a new commercial ELISA using monoclonal antibodies. *Haemophilia* 1998; **4**: 98–103.
20 Verbrugen B, Novakova I, Wessels H, *et al.* The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. *Thromb Haemost* 1995; **73**: 247–51.
21 Giles AR, Verbruggen B, Rivard GE, *et al.* A detailed comparison of the performance of the standard versus the Nijmegen modification of the Bethesda assay in detecting factor VIII:C inhibitors in the haemophilia A population of Canada. Association of Hemophilia Centre Directors of Canada. Factor VIII/IX Subcommittee of Scientific and Standardization Committee of International Society on Thrombosis and Hemostasis. *Thromb Haemost* 1998; **79**: 872–5.
22 Verbruggen B, van Heerde W, Novakova I, *et al.* A 4% solution of bovine serum albumin may be used in place of factor VIII:C deficient plasma in the control sample in the Nijmegen Modification

of the Bethesda factor VIII:C inhibitor assay. *Thromb Haemost* 2002; **88**: 362–4.

23 Mondorf W, Klinge J, Luban NL, *et al*. Low factor VIII recovery in haemophilia A patients without inhibitor titre is not due to the presence of anti-factor VIII antibodies undetectable by the Bethesda assay. *Haemophilia* 2001; **7**: 3–9.

24 Mondorf W, Ehrenforth S, Vigh Z, *et al*. Screening of F.VIII:C antibodies by an enzyme-linked immunosorbent assay. *Vox Sang* 1994; **66**: 8–13.

25 Lindgren A, Wadenvik H, Tengborn L. Characterization of inhibitors to F VIII with an ELISA in congenital and acquired haemophilia A. *Haemophilia* 2002; **8**: 644–8.

26 Shetty S, Ghosh K, Mohanty D. ELISA for factor VIII antibodies: does it detect antibodies much before the conventional Bethesda assay? *Haemophilia* 2003; **9**: 654.

39 Standardization of assays in hemophilia

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Introduction

Standardization, in the context of biologic assays, is a set of procedures to ensure that assays carried out on a sample in one laboratory at any one time will give the same results when carried out in the same laboratory at a different time, or in a different laboratory. In this chapter, the principles and practice of standardization as applied to assays of coagulation factors will be described; the main emphasis is on factor VIII (FVIII) assays, inhibitor assays, and assays for bypassing agents—which are the most frequently performed and present the most problems—but assays of factor IX (FIX), von Willebrand factor (VWF), and factors of the rarer coagulation deficiencies are also covered.

Principles of biologic standardization

Comparative bioassay

Coagulation factor assays are based on the principle of comparative bioassay, in which the observed results on test samples are compared with those of a standard of known potency. By testing different dilutions of the standard, a "dose–response" curve (usually converted to a straight line by mathematical manipulation) can be constructed, and the potency of the test sample can then be obtained by interpolation. The basic assumption implicit in this method is that the test sample behaves exactly like a dilution of the standard. In order to test this assumption, results for the test sample should be obtained at different dilutions. Ideally, at least three dilutions of both standard and test sample should be measured; when concentrations are expressed on a log scale, the lines for test and standard should then be parallel, and the potency can be determined from the distance between the lines. Because of possible errors in dilution and measurement, and the intrinsic biologic variability of the assay system, both the dilutions and the measurements should be repeated, and the results subjected to statistical analysis. A full treatise on statistical analysis of bioassays is given by Finney [1], and a simple computer program for coagulation factor assays is described by Kirkwood and Snape [2].

Assays of concentrates by manufacturers and control laboratories usually follow the above principles, with several independent assays in order to maximize precision. However, many clinical laboratories only test patients' samples at a single dilution, a practice encouraged by the software on modern coagulometers. This practice can give only an imprecise estimate of potency and should be avoided. All clinical samples should be tested with at least two different dilutions, and each dilution should be repeated, giving a minimum of four observations for each test sample, and, wherever possible, a parallel line analysis should be performed.

Standards and units

The unit of activity for almost all coagulation factors was originally defined as the amount in 1 mL of "average normal plasma." This has the great advantage of clinical convenience, but it is clear that "average normal plasma" collected in different laboratories and at different times is unlikely to be the same. Indeed, when the first international collaborative study on FVIII was carried out, samples of pooled normal plasma in the 20 laboratories differed by up to a factor of two [3]. The only way that measurements in different laboratories can be standardized is for the various local or commercial standards used in the assays to be calibrated against stable reference standards with a fixed value.

The establishment of a range of international standards for measurements of biologic activity began as long ago as 1925 with insulin. The first international standard for a coagulation factor (FVIII) was established in 1971 [3] and, subsequently, international standards have been established for all the major coagulation factors (Table 39.1).

Establishment of international standards is the responsibility of the World Health Organization (WHO), and the work on preparation, maintenance, and distribution of these standards is carried out by the National Institute for Biological Standards and Control (NIBSC).

Because of the need to conserve stocks over a long time period, these standards cannot be used routinely in assays; they are intended to calibrate local and manufacturers'

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Table 39.1 International and working standards for coagulation factors.

	Type of standard		Type of material			
Name	IS	WS	Pl	Co	Pu	Code
Factor II	3rd		✓			99/826
	3rd			✓		98/590
		*6th		✓		07/326
Factor V	1st		✓			03/116
Factor VII	3rd		✓			99/826
	1st			✓		97/592
Factor VIIa	2nd				✓	07/228
Factor VIII	8th			✓		07/350
	6th		✓			07/316
		*12th		✓		02/122
Factor IX	3rd		✓			99/826
	4th			✓		07/182
		*6th		✓		07/326
Factor IXa	1st			✓		97/562
Factor X	3rd		✓			99/826
	3rd			✓		98/590
		*6th		✓		07/326
Thrombin	2nd				✓	01/580
Fibrinogen	2nd		✓			98/612
	1st			✓		98/614
Antithrombin	2nd		✓			93/768
	3rd			✓		06/166
Factor XI	1st		✓			04/102
Protein C	2nd		✓			02/342
	1st			✓		04/252
Protein S	2nd		✓			03/228
VWF	1st			✓		00/514
	6th		✓			07/316
Factor XIII	1st		✓			02/206
FEIBA (aPCC)		**1st		✓		06/172

Co, concentrate; IS, international standard; Pl, plasma: Pu, purified; WS, working standard (*British Working Standard; **NIBSC Working Standard).

standards. For FVIII and FIX concentrates, working standards, calibrated against the WHO standards, are available from the Food and Drug Administration Center for Biologics Evaluation and Research (FDA/CBER) and the European Pharmacopoeia (EP). Working concentrate standards are also available for prothrombin (FII), FVIII, FIX, factor X (FX), and FEIBA (Factor Eight Inhibitor Bypassing Activity) from NIBSC (Table 39.1). Plasma standards for most coagulation factors are available commercially.

"Like versus like"

The reason why there are separate plasma and concentrate standards for most coagulation factors is that, as in all biologic assays, variability is minimized when the standard and test are of similar composition. This is not just a theoretical principle; it has been shown to work best in practice. Thus, when FVIII concentrates have been assayed against plasma, or vice versa, variability between laboratories has always been higher than for plasma versus plasma and concentrate versus concentrate assays [4,5].

There are two situations in which this principle is difficult to maintain. First, as is the case with FVIII, there may be a wide diversity of concentrates manufactured, so that even with a concentrate standard there may be substantial differences in composition between test and standard. Second, although postinfusion samples, being plasma from patients, are normally assayed against a plasma standard, in composition they resemble concentrates "diluted" in the patient's plasma, so there may be an argument for using concentrate standards instead. These points are dealt with more fully in subsequent sections.

Standardization of factor VIII assays

Assay methods

The three methods that are used to assay FVIII are the one-stage and two-stage assays, both of which are clotting assays, and the chromogenic method. The principles and practice of these methods are reviewed in detail in Chapter 38.

The one-stage method has always been preferred by clinical laboratories because of its simplicity and ease of automation. The two-stage method was used extensively by manufacturers in the past because of its high precision and independence of supply of deficient plasma. At present, however, the two-stage method is rarely used in clinical laboratories and by only a few manufacturers, but the chromogenic method, which is based on the same principle as the two-stage assay, is being adopted increasingly for assays of concentrates. The EP reference method for concentrates was the two-stage assay, but in 1995 this was replaced with the chromogenic method, which is also the method recommended by the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) [6].

The one-stage method suffers from an extreme diversity in the reagents and assay conditions used; for instance, in clinical laboratories in the UK, 27 different aPTT reagents, 19 different deficient plasmas, 22 different reference plasmas, and 28 different instruments are used [source: UK National External Quality Assessment Scheme (NEQAS) data—Survey 173]. The chromogenic method has less variety, with only five different kits from four manufacturers being available. General comparisons between the methods have been reviewed extensively elsewhere [7,8].

Currently, most European manufacturers of concentrates use the chromogenic method to label their products, whereas most US concentrates, including the two full-length

recombinant products, are assayed by the one-stage method. The BDD recombinant product, ReFacto®, is currently assayed by the chromogenic method.

Assays of concentrates

Potencies of FVIII concentrates are measured in international units (IU), which are defined by the international standard (IS). As indicated in Table 39.2, the WHO standard has been replaced at approximately 5-year intervals. This is partly because of high demand, but also because of changes in the methods of production of clinical concentrates over time, and the need for the WHO standard to be representative of current concentrates. The current WHO IS for FVIII concentrate is the seventh IS (99/678) and is a plasma-derived material [9]; however, this is shortly to be replaced by the recently established WHO 8th IS (07/350).

The large differences in composition among current plasma-derived and recombinant products has meant that the "like versus like" principle is increasingly difficult to apply and, despite the universal use of the WHO standard as the primary reference material for concentrates, discrepancies between methods, and between different laboratories using the same method, can still occur [8]. However, the reasons for some of these discrepancies have been established, and recommendations for assays of concentrates were developed by ISTH/SSC [6].

These recommended assay conditions were used in an international collaborative study to calibrate the sixth WHO concentrate standard, a recombinant material, and the inter-laboratory coefficients of variation (CVs) overall were 9–10% [5]. Thus, it is clear from these studies that, provided certain technical requirements are adhered to, recombinant FVIII (rFVIII) concentrates can be assayed satisfactorily against plasma-derived concentrate standards, and there is no need for separate standards for the two types of products. The question of whether plasma-derived products can be assayed satisfactorily against a rFVIII standard was addressed in a study by Abertengo *et al*. [10], where nine different plasma-derived products were assayed against both the fifth (plasma-derived) and sixth (recombinant) WHO standards. There were no significant differences in potencies using the two standards, indicating that a recombinant standard can be satisfactory for calibration of a wide variety of plasma-derived concentrates.

Table 39.2 World Health Organization factor VIII concentrate standards.

Number	Purity and type	Year established	Calibrated against
1	Low (PD)	1971	"Normal plasma"
2	Low (PD)	1978	1st IS
3	Intermediate (PD)	1983	2nd IS
4	Intermediate (PD)	1989	3rd IS
5	High (PD)	1994	4th IS
6	Recombinant	1999	5th IS concentrate and 4th IS plasma
7	High (PD)	2003	5th and 6th IS concentrates, EP2 and Mega I US concentrate standard
8	High (PD)	2009	7th IS EP3 and Mega II US

PD, plasma derived; IS, international standard.

Comparison of methods on concentrates

The use of concentrate standards for assay of therapeutic concentrates follows the "like versus like" principle, and should theoretically result in potencies that are similar by the three different FVIII methods. Although this was broadly the case in the 1970s and early 1980s, developments in viral inactivation methods and the introduction of high-purity plasma-derived and recombinant products increased the diversity of composition of concentrates, and this has led to discrepancies between methods, even though concentrate standards are used [11]. Hubbard *et al*. [12] compared potency estimates by the one-stage and chromogenic methods in 10 different concentrates (seven plasma-derived and three recombinant). The largest discrepancies were associated with a plasma-derived immunopurified concentrate, where the mean one-stage potency exceeded the chromogenic potency by 33%, and with a BDD recombinant product, where the chromogenic potency exceeded the one-stage potency by 28%.

Comparisons of methods on full-length recombinant products have given varied results. Table 39.3 summarizes the data

Table 39.3 Comparison of methods on recombinant concentrates: data from controlled collaborative studies.

Study date	WHO reference standard	Test sample	Potency (%)		
			One-stage	Two-stage	Chromogenic
1992	4th IS	1	100 (9)	99.7 (5)	114.6 (10)
		2	100 (9)	91.2 (5)	98.5 (10)
1994	4th IS	3	100 (17)	104.1 (6)	110.6 (18)
	5th IS	3	100 (17)	101.5 (6)	118.3 (18)
1998	5th IS	4	100 (21)	94.7 (6)	110.7 (25)
		5	100 (21)	93.8 (6)	110.5 (25)
2003	5th IS	6	100 (12)	–	104.3 (14)
	7th	6	100 (26)	–	101.2 (31)

Samples 1–6 are ampouled preparations of two different full-length recombinant Factor VIII concentrate products. Figures in parentheses represent number of laboratories performing each method. Potencies have been calculated as a percentage of those with the one-stage method. All participants used the SSC-recommended methodology and data were analyzed centrally.
WHO, World Health Organization.

on potencies of recombinant concentrates from four "controlled" collaborative studies in which a common standard, defined protocol, and central calculation of results were used. It can be seen that, for seven of the eight data sets, the chromogenic assays gave mean potencies higher than those obtained using the one-stage method, by amounts ranging from 1% to 18%. It should be borne in mind that, at the time of the first two of these studies, the chromogenic method had only recently been introduced for the assay of concentrates in several laboratories, and data from the most recent study may be more relevant to the current situation. Overall, it appears that, particularly if ISTH-recommended methodology is used, the differences in potency of full-length recombinant products between one-stage and chromogenic methods are less than 10% and are unlikely to be clinically significant.

The situation with the BDD concentrate ReFacto is, however, less satisfactory. Mikaelsson *et al*. [13] found that, even when using the WHO concentrate standard and ISTH/SSC-recommended methodology, the potency of this concentrate was substantially higher by the chromogenic method than by the one-stage method, by up to a factor of two depending on the reagents used. Further studies showed that this discrepancy was a result of the type and concentration of phospholipid reagent used in the one-stage method, and comparison with assays in the presence of platelets, and with *in vivo* recovery data, showed that the chromogenic potency was the appropriate value. The chromogenic method is therefore used by the manufacturers to label this product. However, recent studies have shown that, in assays of ReFacto against concentrate standards, either plasma-derived or full-length recombinant, differences of up to 20% may be found between results with different chromogenic kits [14]. These results emphasize the need to pay careful attention to methodology when assaying FVIII concentrates with different molecular composition to that of normal FVIII.

Field studies, carried out on behalf of the ISTH/SSC over time, perhaps give a truer picture as to how assays perform routinely in laboratories, where local methodologies, standards, and calculation of results are used. Such studies have revealed large assay discrepancies and high interlaboratory variability with CVs generally much greater than observed in controlled studies [15]. Figure 39.1 illustrates that, in general, rFVIII and intermediate purity (IP) plasma-derived FVIII tend to give lower one-stage potencies compared with the chromogenic method (though not significantly so for the BDD rFVIII on this occasion), whereas high-purity plasma-derived FVIII tend to give higher one-stage potencies compared with the chromogenic method. In both cases, this discrepancy is significantly reduced when following the ISTH/SSC recommendation of using FVIII-deficient plasma as prediluent [6]. An exception to this was for a product manufactured by the "Method M" monoclonal antibody process where discrepancy of up to 24% was observed between one-stage and chromogenic methods. It has been suggested that differences

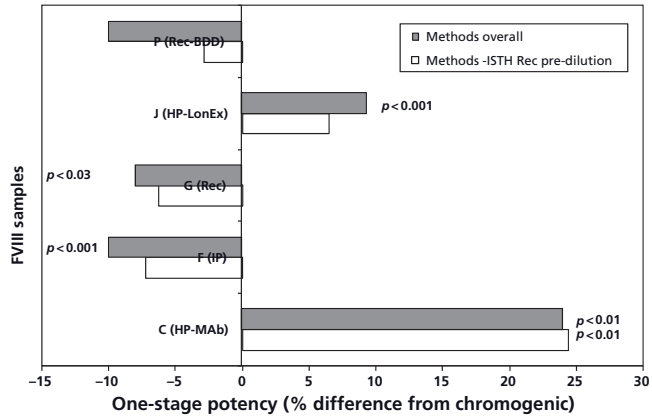

Figure 39.1 Bar chart illustrating potency discrepancies observed in "field" studies between one-stage and chromogenic methods, and the effect of using the ISTH/SSC-recommended FVIII-deficient plasma as prediluent. One-stage potencies are expressed as percentage difference from chromogenic potencies.

Table 39.4 Comparison of methods on potency assessment of recombinant concentrates: data from ISTH/SSCSSC/8 "field" collaborative studies.

	Potency (%) overall		Potency (%) laboratories prediluting in FVIII-deficient plasma	
Test sample	One-stage	Chromogenic	One-stage	Chromogenic
T (FL)	100 (12)	113.8* (26)	100 (5)	111.7 (24)
V (BDD)	100 (12)	118.4* (26)	100 (5)	122.5* (24)
W (FL)	100 (12)	106.1 (26)	100 (5)	102.4 (24)

Samples T and W are two different full-length (FL) recombinant factor VIII products; sample V is a B-domain-deleted (BDD) recombinant FVIII product. Figures in parentheses represent the number of laboratories performing each method. Potencies have been calculated as a percentage of those with the one-stage method. *Significant differences ($P < 0.05$) in potency between the two methods.

in thrombin activation between the two methods may play a role in these types of discrepancies, and evidence for this has been found in recent studies by Hubbard *et al*. [16].

A more recent field study on rFVIII concentrates [17] revealed that although using FVIII-deficient plasma prediluted reduced the discrepancy in potency between one-stage and chromogenic methods for full-length rFVIII products, this was not true for the BDD material (Table 39.4), unlike that observed in the previous field study [15]. Furthermore, although the ISTH/SSC recommendation of using the chromogenic method and prediluting in deficient plasma tended to reduce the interlaboratory variability, the largest reduction in variability (with CVs down to the levels seen in controlled

Table 39.5 Comparison of interlaboratory variability on recombinant FVII concentrates: data from ISTH/SSC "field" collaborative studies.

	Geometric coefficient of variation (%GCV) for recombinant test samples		
Study methods	T (FL)	V (BDD)	W (FL)
SSC/8 overall (one-stage and chromogenic)	14.6 (38)	19.2 (38)	14.5 (38)
SSC/8 ISTH/SSC recommended method (chromogenic + predilution in FVIII-deficient plasma)	12.3 (24)	13.6 (24)	14.4 (24)
SSC/9 ISTH/SSC recommended method (chromogenic + predilution in single batch of FVIII-deficient plasma)	6.8 (25)	7.8 (25)	10.8 (25)

Samples T and W are two different full-length (FL) recombinant factor VIII products; sample V is a B-domain-deleted (BDD) recombinant FVIII product. Figures in parentheses represent the number of laboratories performing each method.

studies) was in fact because of the use of a single batch of FVIII-deficient plasma for predilution (Table 39.5). This suggests that quality and type of FVIII-deficient plasma, as well as variation between manufacturers and even batch-to-batch variation of FVIII-deficient plasma, may be critical in assessing FVIII potency.

Assays of plasma

The establishment of plasma standards has been more straightforward than for concentrates. Here, the main issue has been to maintain the link between the international unit and the "normal plasma unit;" this has been done by incorporating samples of normal plasma pools in the calibration studies for each successive international standard, and adjusting the potencies where necessary to minimize the difference between the IU and "1 mL average normal plasma" (e.g., in the calibration of the fourth IS for FVIII/VWF plasma [18]).

Despite the widespread use of the WHO standard to calibrate local and commercial working standards, the variability in FVIII assays among clinical laboratories in the UK has remained disappointingly high, with CVs of up to 30% in successive NEQAS surveys. This is probably because of the great variety of reagents and methodology in the different laboratories, as referred to earlier; the widespread practice of using only single dilutions may also be a contributory factor.

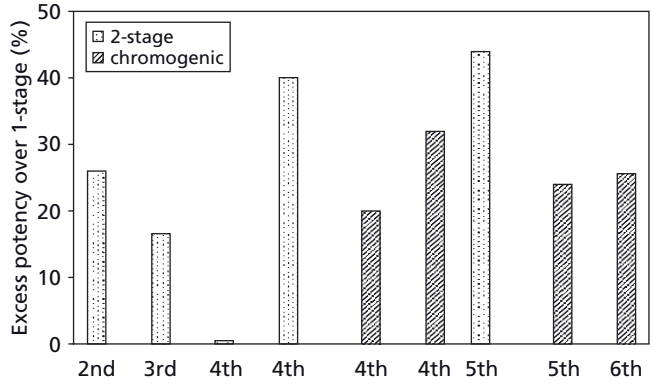

Figure 39.2 Comparisons of assay methods for World Health Organization (WHO) concentrate standards assayed against WHO plasma standards. Data are means from large international collaborative studies, and potencies with the two-stage and chromogenic methods are calculated as percentages of the one-stage potencies. Results are plotted as percentage differences (e.g. 20% for the chromogenic method means the potency using that method is 120% of the one-stage potency).

Postinfusion plasma

The assay of concentrates against plasma standards has been a longstanding problem because of wide variability between laboratories and a basic difference between assay methods, and for this reason two separate WHO standards for plasma and concentrates were developed. However, although such comparisons are avoided in routine assays, they are relevant to manufacturers of plasma-derived concentrates, and especially to clinicians measuring *in vivo* recovery. In the latter situation, patients' postinfusion samples, which essentially consist of concentrates "diluted" in the patient's hemophilic plasma, are assayed against a plasma standard.

In vitro assays of concentrates versus plasma standards have almost invariably given higher potencies by two-stage or chromogenic methods than by the one-stage method. Figure 39.2 summarizes the data from several collaborative studies over a period of 15 years, and the consistency of the trend is clearly seen, with only one of 10 data sets giving no discrepancy. Despite considerable investigation, the basic causes of this discrepancy remain unknown, although it is thought that the extensive processing applied to both plasma-derived and recombinant concentrates could lead to differences in their rates of activation and inactivation in the two method types from the FVIII in normal plasma; there is some evidence for this from recent studies [19]. For largely historical reasons, when the WHO concentrate and plasma FVIII standards are compared against each other, the values are approximately equivalent by one-stage assays but not by two-stage or chromogenic methods, as shown in Figure 39.2.

These figures help to explain the large discrepancies between chromogenic and one-stage potencies found in patients' samples after infusion of recombinant concentrates [20]. It appears that, after infusion, the recombinant products are

behaving in an essentially similar manner in these assays to samples produced by diluting them *in vitro* in hemophilic plasma.

The situation with plasma-derived products is variable, dependent on the nature of the product and the test systems used. For instance, in a study by Lee *et al*. [21], Hemofil M® was found to give a 20% discrepancy in postinfusion plasmas between one-stage and chromogenic methods, whereas in a study of similar products performed at Sanquin Laboratories there was no difference between the methods. Equivalence between the methods was also found in a UK NEQAS study on a postinfusion sample from a different type of plasma-derived concentrate.

A practical solution to this problem that has been discussed by the FVIII/FIX Subcommittee of ISTH/SSC is to regard the postinfusion samples as concentrates, "diluted" in a patient's plasma, which is essentially what they are, and use a concentrate standard, diluted in hemophilic plasma, instead of a plasma standard, to construct the standard curve. This approach has recently been tested in an *in vivo* recovery study, in which patients' samples after infusion of Recombinate were assayed against both a plasma standard and a concentrate standard consisting of Recombinate itself [20]. The results showed that the 25% discrepancy between one-stage and chromogenic methods using the plasma standard was completely abolished with the concentrate standard. In a field study, the use of a ReFacto concentrate standard was similarly associated with a reduced variability between laboratories and an abolition of the discrepancy between one-stage and chromogenic assays [22].

Standardization of factor IX assays

Standardization of FIX assays has presented fewer problems than that of FVIII. This is because, apart from the very recent introduction of a chromogenic method, only a single assay method, the one-stage clotting assay, has been used for both plasma and concentrates. As for FVIII, a concentrate standard was the first to be established by WHO, for therapeutic materials, and this consisted of a prothrombin complex concentrate (PCC) [23]. The switch to high-purity single FIX concentrates as the mainstay of therapy does not appear to have caused any problems in assay standardization; it appears that PCCs and single FIX concentrates can be assayed satisfactorily against each other. The current WHO standard is a single FIX concentrate, and the same material also serves as the FDA and EP standards. However, this standard is shortly to be replaced by the recently established WHO 4th IS (07/182). This material will also serve as the FDA and EP standards.

As for FVIII, it was found that predilution of concentrates in FIX-deficient plasma was necessary to obtain optimum and reproducible potency when assaying concentrates against a concentrate standard, and also when comparing concentrates against plasma [24]. An international plasma standard for FIX, together with the other vitamin K-dependent factors, II, VII, and X, was established by WHO in 1987 [25], and most local and commercial plasma standards are now calibrated in international units. However, UK NEQAS surveys continue to show wide variability between laboratories, probably because of the multiplicity of aPTT reagents and deficient plasmas used. As with FVIII, artificially depleted plasmas have become the main type of FIX-deficient reagent used, but there has been no systematic study of their performance compared with hemophilia B plasma.

Standardization of inhibitor assays

Development of neutralizing antibodies (inhibitors) to FVIII (or FIX) is a serious complication of hemophilia treatment. Accurate measurement of inhibitors is important for determining the appropriate treatment for this complication. However, poor reproducibility of inhibitor assays in different laboratories has been recognized since the early days of hemophilia treatment, and a major step forward was the introduction of the "Bethesda assay" in 1975 [26]. This method standardized the conditions for incubation of the inhibitor sample with a source of FVIII (2 h at 37°C with pooled normal plasma as the FVIII source), though measurement of residual FVIII continued to be performed using local methodology. The "Bethesda Unit" of FVIII inhibitory activity was defined as the amount of inhibitor that gives 50% reduction of FVIII under the defined assay conditions.

Despite this commendable attempt at standardization, a collaborative study in 1982 showed very high variability in inhibitor assays among laboratories, with CVs ranging from 33% to 202% [27]. However, the ranking of the various inhibitor samples was similar in the different laboratories and it was suggested that an inhibitor standard might improve the situation. A study (3-day workshop) carried out at NIBSC in 1985 showed that the high variability observed with inhibitor assays can be reduced by standardizing the different stages of an inhibitor assay [28] and that this variation can be further reduced by assaying relative to an inhibitor standard, see Table 39.6. A further improvement in standardization of inhibitor assays—the Nijmegen modification—was described by Verbruggen and colleagues [29]. In this method, FVIII-deficient plasma was substituted for buffer in the control mixture, and the normal plasma was also buffered. This method was shown to be more reliable at low concentrations, and is now recommended by the ISTH/SSC [30]. In a recent European external QC survey, however, the interlaboratory CVs were still high: 48% for the Bethesda method and 32% for the Nijmegen modification [31].

The possible development of an inhibitor standard was investigated in a number of international collaborative studies by Raut *et al*. [32,33]. In the preliminary studies it appeared that comparison of inhibitor samples against a putative standard did give some improvement in agreement between

Table 39.6 Summary of data from NIBSC inhibitor workshop. The effect of an inhibitor reference standard antibody on the variability of inhibitor assays.

Session	Different standardized stages in the inhibitor assay	CVs (%) no reference standard	CVs (%) reference standard
1	Assays of samples containing no inhibitors	15–26	–
2	Assays of samples containing inhibitors	53–80	20–30
3	Assays of samples containing inhibitors when incubation stage standardized	33–43	13–34
4	Assays of samples containing inhibitors when incubation + FVIII assay stages standardized	14–20	6–29

Consistent improvement in %CV (coefficient of variation) when assayed relative to a inhibitor reference standard.

laboratories [32]. In a more recent larger study, when nine inhibitor samples were assayed in 22 laboratories, the interlaboratory variability was surprisingly low, with CVs of 17–33% [33]. Although this remained higher than in studies of noninhibitor samples, it nevertheless represented the best interlaboratory agreement ever achieved for an inhibitor study to date. Comparison of inhibitor samples against one putative standard further reduced the interlaboratory variability. The highest variability was associated with assays of low-titer inhibitor samples, indicating that sensitivity of this assay at low inhibitor titers needs to be improved. A further explanation for the high variability is that the inhibitor can continue to act on FVIII during the assay of residual FVIII; therefore, FVIII assay methods that have short incubation times are likely to give the truest measure of inhibitor titer. There is some evidence that chromogenic methods, which mostly do have short incubation times, give less variability of inhibitor titer [32,33], but this needs to be confirmed in a larger number of laboratories. The study concluded that further investigations into the reasons for the relatively high interlaboratory variability were needed before a reference standard could be established for this very complex area of measurement.

Standardization of von Willebrand factor assays

Assays for von Willebrand factor

As described in Chapter 44, the ristocetin cofactor method has continued to be the mainstay of assays of VWF, but it suffers from very high variability, both within and between laboratories. There are difficulties also with preparation and stability of the platelet reagent, and the assay is labor-intensive and difficult to automate. Because of these problems, alternative methods of measuring VWF function have been sought, and the collagen-binding method, which is much easier to perform and more reproducible, was introduced by Favorolo and colleagues [34]. Although there has been some controversy over its clinical applicability, it is used in a number of clinical laboratories in conjunction with other methods such as multimer analysis, and is being considered also as a possible EP reference method for concentrates (see below). The measurement of VWF antigen is also useful as an adjunct to the functional measurements; the original "rocket" electrophoresis method of Laurell has now been largely replaced by ELISA assays.

Standards for von Willebrand factor

The course of standardization of VWF differed from that of FVIII and FIX in that, when the first International Standard for FVIII, plasma, was established in 1982, the same plasma was calibrated (against normal pools) for VWF [35]. Thus, a plasma standard was established before a concentrate standard, and in fact a separate concentrate international standard for VWF concentrate was established only recently [36]. The reason for this is the relatively slow development and licensing of VWF concentrates, with cryoprecipitate and FFP continuing to be used for many years.

Standardization of bypassing agents

Activated prothrombin complex concentrates

FEIBA® is an activated prothrombin complex concentrate (aPCC) produced by Baxter Healthcare in Austria, which is used to control spontaneous bleeding episodes or cover surgical interventions in hemophilia patients who develop inhibitors [37]. The *in vitro* assay for FEIBA measures the ability of this product to shorten the activated partial thromboplastin time (aPTT) of a FVIII-deficient plasma containing FVIII inhibitors. FVIII inhibitor bypassing activity (FEIBA) is expressed in arbitrary units. One unit of FEIBA was originally defined as that amount of FEIBA that shortens the aPTT of high-titer FVIII inhibitor reference plasma to 50% of the blank value. There is a linear correlation between the logarithm of the FEIBA and the logarithm of the measured clotting time. The quantitative evaluation is made using a parallel line assay, or by using a calibration curve, relative to the manufacturer's own reference standard. These standards are produced by the manufacturer on a relatively small scale and are frequently replaced. They are generally calibrated relative to their predecessor. More recently, following concerns about the reliability of this procedure, it was decided to develop a separate independent FEIBA reference standard (in relatively large quantities); this was established as a first

NIBSC Working Standard for FEIBA after a collaborative study involving the manufacturers and three European control laboratories [38].

Clinical monitoring of patients undergoing FEIBA bypassing therapy, which would reflect achievement of hemostasis *in vivo*, has been difficult, although a thrombin generation assay [39] recently developed seems to show some promise. Nevertheless, a truly standardized assay for *ex vivo* monitoring has still been elusive.

Factor VIIa

Recombinant activated factor VII (rFVIIa; NovoSeven®, Novo Nordisk, Denmark) has proved very successful in the treatment of inhibitor patients, although there are some patients who do not respond. The product was initially assayed against a plasma standard for FVII, but these assays proved quite variable, and a separate international standard for FVIIa was developed [40].

FVIIa has a short half-life and considerable interindividual pharmacokinetic variability; consequently, many clinicians argue that treatment should be monitored, though the usefulness and practicality of such monitoring remain topics for debate. The injected FVIIa leads to a considerable increase in the patient's FVII clotting activity (FVII:C), and routine FVII:C assays can be used for monitoring. However, a specific assay for FVIIa has been developed by Morrissey *et al*. [41] and modified by Johannessen *et al*. to make it more reliable at low concentrations [42]. The reliability and clinical utility of FVII:C and FVIIa assays in monitoring rFVIIa treatment has been recently reviewed [43].

Standardization of assays of other coagulation factors

Assays of other coagulation factors are carried out only rarely in the context of congenital deficiencies, although they are performed more frequently in relation to acquired defects, population surveys, and by manufacturers of concentrates. In general, the same principles apply, as for FVIII and FIX, with plasma standards being used for plasma samples, and concentrate standards for assay of therapeutic concentrates (see Table 39.1).

Standardization of global assays

In recent years there has been increasing recognition that specific assays may not be sufficiently informative to assess the patient's overall hemostatic state and that global tests may contribute useful additional information. Another reason for the revival of global assays is the development of technical innovations and more sophisticated methods of analysis, which have improved their reliability and reproducibility and led to more widespread use. Two tests developed in the 1950s that have recently undergone a renaissance are thromboelastography and the thrombin generation test (TGT). Both methods have been investigated for the assessment of patients with hemophilia; the TGT has been used more widely and appears to show the most promise.

Interest in the TGT was rekindled following a study by Hemker [44], in which the method was modified by the use of a chromogenic substrate to monitor the generated thrombin. This and other technical modifications provided much-improved precision and sensitivity [45]. Subsequently, there have been other technical variations, notably the development of different chromogenic and fluorescent substrates that can be incorporated into the incubation mixture to give enhanced convenience and sample throughput [46]. Use of TGT for measuring activity of FVIII concentrates was investigated by McIntosh *et al*. [47]. Collaborative studies of the TGT on hemophilic patients' samples are currently being planned, and at present it is too early to give any recommendations regarding standardization of this test.

References

1 Finney D. *Statistical Method in Biological Assay*. London: Charles Griffin, 1978.
2 Kirkwood TBL, Snape TJ. Biometric principles in clotting and clot lysis assays. *Clin Lab Haematol* 1980; **2**: 155–67.
3 Bangham DR, Biggs R, Brozovic M, *et al*. A biological standard for measurement of blood coagulation factor VIII activity. *Bull WHO* 1971; **45**: 337–51.
4 Kirkwood TBL, Barrowcliffe TW. Discrepancy between 1-stage and 2-stage assay of factor VIII:C. *Br J Haematol* 1978; **40**: 333–8.
5 Raut S, Heath A, Barrowcliffe TW. Establishment of the 6th International Standard for FVIII concentrate. *Thromb Haemost* 2001; **85**: 1071–8.
6 Barrowcliffe TW. Factor VIII and Factor IX Sub-committee. Recommendations for the assay of high-purity factor VIII concentrates. *Thromb Haemost* 1993; **70**: 876–7.
7 Barrowcliffe TW. The one-stage versus the two-stage factor VIII assay. In: Triplett DA (ed.) *Advances in Coagulation Testing*. Skokie, IL: College of American Pathologists, 1986: 47–62.
8 Hubbard AR, Curtis AD, Barrowcliffe TW, *et al*. Assay of factor VIII concentrates: comparison of chromogenic and 2-stage clotting assays. *Thrombosis Res* 1986; **44**: 887–91.
9 Raut S, Bevan S, Hubbard AR, Sands D, Barrowcliffe TW. A collaborative study to establish the 7th International Standard for Factor VIII Concentrate. *J Thromb Haemost* 2005; **3**: 119–26.
10 Albertengo ME, Barrowcliffe TW, Oliva L, Bevan S, Raut S. New recombinant standard for FVIII concentrate gives same results as previous plasma derived standards on a range of FVIII products. *Thromb Haemost* 2000; **83**: 789–90.
11 Barrowcliffe TW, Watton J, Tubbs JE, *et al*. Potency of high purity factor VIII concentrates (Letter). *Lancet* 1990; **ii**: 124.
12 Hubbard AR, Weller LJ, Bevan SA. A Survey of one-stage and chromogenic potencies in therapeutic FVIII concentrates. *Br J Haematol* 2002; **117**: 247–8.

13 Mikaelsson M, Oswaldsson U, Sandberg H. Influence of phospholipids on the assessment of factor VIII activity. *Haemophilia* 1998; **4**: 646–50.
14 Hubbard AR, Sands D, Sandberg E, *et al*. A multi-centre collaborative study on the potency estimation of ReFacto. *Thromb Haemost* 2003; **90**: 1088–93.
15 Raut S, Sands D, Heath AB, Barrowcliffe TW. Variability in factor VIII concentrate measurement: results from SSC field collaborative studies. *J Thromb Haemost* 2003; **1**: 1927–34.
16 Hubbard AR, Weller LJ, Bevan SA. Activation profiles of FVIII in concentrates reflect one-stage/chromogenic potency discrepancies. *Br J Haematol* 2002; **117**: 957–60.
17 Raut S, Heath AB, Lee M, Mertens K. Collaborative study on recombinant factor VIII concentrates: SSC/8/9—phase I & II field studies. Report to participants. An SSC working party study on behalf of the SSC FVIII/IX subcommittee. Unpublished report, 2007.
18 Hubbard AR, Rigsby P, Barrowcliffe TW. Standardisation of Factor VIII and von Willebrand Factor in plasma: Calibration of the 4th International Standard (97/586). *Thromb Haemost* 2001: **85**: 634–8.
19 Hubbard AR, Bevan SA, Weller LJ. Potency estimation of recombinant factor VIII: effect of assay method and standard. *Br J Haematol* 2001; **113**: 533–6.
20 Lee CA, Owens D, Bray G, *et al*. Pharmacokinetics of recombinant factor VIII (Recombinate) using one-stage clotting and chromogenic factor VIII assay. *Thromb Haemost* 1999; **82**: 1644–7.
21 Lee CA, Barrowcliffe TW, Bray G, *et al*. Pharmacokinetic in vivo comparison using 1-stage and chromogenic substrate assays with two formulations of Hemofil-M. *Thromb Haemost* 1996; **76**: 950–6.
22 Ingerslev J, Jankowski, M, Weston SB, Charles LA. Collaborative field study on the utility of a BDD factor VIII concentrate standard in the estimation of BDD factor VIII:C activity in hemophilic plasma using one-stage clotting assays. *J Thromb Haemost* 2004; **2**: 623–8.
23 Brozovic M, Bangham DR. Study of a proposed International Standard for factor IX. *Thromb Haemost* 1976; **35**: 222–36.
24 Barrowcliffe TW, Tydeman MS, Kirkwood TBL. Major effect of prediluent in factor IX clotting assay. *Lancet* 1979; **ii**: 192.
25 Barrowcliffe TW. Standardisation of factors II, VII, IX, and X in plasma and concentrates. *Thromb Haemost* 1987; **59**: 334.
26 Kasper CK, Aledort L, Aronson D, *et al*. Proceedings: A more uniform measurement of factor VIII inhibitors. *Thromb Diath Haemorrh* 1975; **34**: 612.
27 Austen DE, Lechner K, Rizza CR, Rhymes IL. A comparison of the Bethesda and New Oxford methods of factor VIII antibody assay. *Thromb Haemost* 1982; **47**: 72–5.
28 Barrowcliffe TW, Peake IR. Factor VIII Inhibitor Assays: NIBSC 3-Day Workshop. Report to Participants. Unpublished report, 1982.
29 Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. *Thromb Haemost* 1995; **73**: 247–51.
30 Giles AR, Verbruggen B, Rivard GE, Teitel J, Walker I. A detailed comparison of the performance of the standard versus the Nijmegen modification of the Bethesda assay in detecting factor VIII:C inhibitors in the haemophilia A population of Canada. *Thromb Haemost* 1998; **79**: 872–5.
31 Verbruggen B, Meijer P. Performance of Bethesda assay and Nijmegen variant in external quality assessment (ECAT) of factor VIII inhibitor assays. *J Thromb Haemost* 2007; **5**(Suppl. 2): O-T-093.
32 Raut S, Sands D, Kitchen S, Preston FE, Barrowcliffe TW. Collaborative study on determination of FVIII Inhibitors. Report to participants. An SSC working party study on behalf of the SSC FVIII/IX subcommittee. Unpublished report, 2005.
33 Raut S, Faulkner L, Heath A. Collaborative study on proposed reference standard for FVIII Inhibitors. Report to participants. An SSC working party study on behalf of the SSC FVIII/IX subcommittee. Unpublished report, 2007.
34 Favorolo EJ. Collagen binding assay for von Willebrand factor (vWF:CBA): detection of von Willebrand's disease (vWD) and discrimination of vWD subtypes, depends on collagen source. *Thromb Haemost* 2000; **83**: 127–35.
35 Barrowcliffe TW. Standardization and assay. *Semin Thromb Haemost* 1993; **19**: 73–9.
36 Hubbard AR, Sands, Chang A, Mazurier C. Standardisation of von Willebrand factor in therapeutic concentrates: calibration of the 1st International Standard for von Willebrand factor concentrate (00/514). *Thromb Haemost* 2002; **88**: 380–6.
37 Hilgartner MW, Knatterud G, and The FEIBA Study Group. The use of factor-eight-inhibitor-by-passing-activity (FEIBA IMMUNO). Product for treatment of bleeding episodes in haemophiliacs with inhibitors. *Blood* 1983; **61**: 36–40.
38 Raut S, Daniels S, Hockley J, Heath A. An International Collaborative Study to Calibrate the 1st NIBSC Reference Standard for Factor VIII Bypassing Activity (FEIBA). Report to participants, Unpublished report, 2008.
39 Varadi K, Negrier C, Berntorp E, *et al*. Monitoring the bioavailability of FEIBA with a thrombin generation assay. *J Thromb Haemost* 2003; **11**: 2374–80.
40 Hubbard AR, Weller LJ, Heath A. Calibration of the proposed WHO 2nd international standard factor VIIa concentrate (07/228). WHO ECBS Report 2008, WHO/BS/08.2090.
41 Morrissey JH, Macik BG, Neuenschwander PF, Comp PC. Quantitation of activated factor VII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activation. *Blood* 1993; **81**: 734–44.
42 Johannessen M, Nielsen G, Nordfang O. Comparison of the factor VII:C clot analysis and a modified activated factor VII analysis for monitoring factor VII activity in patients treated with recombinant activated factor VII (NovoSeven). *Blood Coagul Fibrinolysis* 2000; **11**(Suppl. 1): S159–64.
43 Barrowcliffe TW. Monitoring inhibitor patients with the right assays. *Semin Hematol* 2008; **45**(Suppl. 1): S25–S30.
44 Hemker HC. The mode of action of heparin in plasma. In: Verstraete M, Vermylen J, Lijnen R, Arnout J (eds.) *Thrombosis and Haemostasis*. Leuven, Belgium: Leuven University Press, 1987: 17–36.
45 Hemker HC, Wielders S, Kessels H, Béguin S. Continuous registration of thrombin generation in plasma, its use for the determination of the thrombin potential. *Thromb Haemost* 1993; **70**: 617–24.
46 Hemker HC, Giesen P, Al Dieri R, *et al*. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb* 2003; **33**: 4–15.
47 McIntosh JH, Owens D, Lee CA, Raut S, Barrowcliffe TW. A modified thrombin generation test for the measurement of factor VIII concentrates. *J Thromb Haemost* 2003; **1**: 1005–11.

40 Global laboratory assays in hemophilia

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Introduction

An immediate consequence of a break in the vascular endothelium is exposure of extravascular tissue factor-bearing cells and platelet adhesion to the damaged vessel wall. Following activation via, for example, epinephrine, adenosine diphosphate (ADP), and collagen, the platelets aggregate and form a surface of negatively charged phospholipids where the coagulation factors can assemble and perform their enzymatic and catalytic functions [1]. The main purpose of the coagulation factors is to produce a timely, sufficient, and regulated amount of thrombin to cleave fibrinogen and activate FXIII and thrombin activatable fibrinolysis inhibitor in order to produce a stable hemostatic plug and stop bleeding. Furthermore, the purpose of the hemostatic plug is to establish a matrix to facilitate repair and regeneration of the integrity of the vessel wall. Thrombin may be considered as the lead protein for stimulating and directing the generation of a hemostatic plug. In other words, thrombin generation may be characterized as the momentum of the hemostatic system. Following production of less than 1% of the total thrombin generated in a healthy hemostatic system, a series of timely coordinated activation processes takes place: platelet activation, activation of factor V and XIII, cleavage of fibrinogen, and activation of factor VIII and factor XI [2]. This initiation phase of thrombin generation is followed by a propagation phase of thrombin driven by the intrinsic tenase (FIXa and FVIIIa) and prothrombinase (FXa and FVa) complexes [1]. The dynamic production of thrombin is regulated by natural anticoagulants, such as antithrombin and proteins C and S in order to prevent excessive production of thrombin that may result in thromboembolic events.

Thrombin is not the only determinant required to achieve hemostasis. Thrombin acts on substrates, such as fibrinogen and platelets, constituting the three-dimensional bricks of the hemostatic plug. Furthermore, hemostasis is also characterized by the formation of a clot structure being resistant to fibrinolysis and with physical strength to withstand blood pressure and flow of blood.

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The hemostatic system is regulated by a coordinated interaction of coagulation proteins, natural anticoagulants, and cells. In particular, the platelets and the endothelium play an important role in the regulation of hemostasis; however, both white and red blood cells play supportive roles and impact thrombin generation and clot formation.

Limitations of standard coagulation assays

Standard coagulation assays such as the activated clotting time (aCT), prothrombin time (PT/INR), or activated partial thromboplastin time (aPTT) provide information only of the initiation of clot formation. However, formation of a sufficient hemostatic plug is a continuous process with characteristic rate-specific properties. When there is a suspicion of hemophilia, the aPTT may be used to screen for potential factor deficiencies. If the aPTT is abnormal, levels of coagulation factor VIII (hemophilia A) or factor IX (hemophilia B) are evaluated by performing a one-stage clotting assay or a chromogenic assay. The one-stage clotting assay is also based on measurements of aPTT utilizing factor VIII-deficient plasma, reference plasma, and patient plasma. Usually, these routine coagulation assays can provide a specific biochemical diagnosis to categorize patients as severe, moderate, or mild. However, the standard aPTT and single coagulation factor assays are only marginally correlated with the clinical phenotype of the coagulation disorder. Thus, some patients with severe hemophilia A (FVIII : C < 1%) bleed frequently, whereas other patients bleed only occasionally despite similar biochemical diagnosis and comparable lifestyle.

The ideal global coagulation assay

The ideal global assay would be one that provides information on the dynamics of thrombin generation; includes all components involved in regulation of hemostasis, such as coagulation proteins, natural anticoagulants, blood cells, and endothelium; demonstrates a visual assessment of whole blood clot formation and/or fibrin formation; and evaluates the structure and stability of the formed clot. In addition, it should be easy to

operate, give rapid and reproducible results and be inexpensive. Furthermore, the ideal global assay should correlate to clinical baseline phenotype and aid in predicting severity of the bleeding pattern. Finally, the assay should be useful for monitoring hemostatic interventions and guide the selection of the most optimal hemostatic drug and necessary dosage to achieve hemostasis. In addition, portability would be a great benefit such that it can be used in the patient care setting.

Currently, such an assay does not exist; however, there are some very attractive candidates available. This chapter gives a description of methods for measuring thrombin generation and whole blood coagulation with thromboelastography/thromboelastometry.

Methodologies

Measurement of continuous thrombin generation

History

In the 1960s and 1970s, the brilliant ideas of researchers such as Biggs, Hemker, and Béguin started a pioneering series of studies on modalities for measurement of thrombin generation. Initially, measurements were based on cumbersome and time-consuming, yet thoroughly performed, subsampling techniques (see ref. 3 for a historical review). It became obvious that automated techniques were needed if measurement of thrombin generation were to be adapted into clinical use. Hence, in the 1990s HC Hemker and colleagues invented calibrated automated thrombin generation measurements in platelet-poor and platelet-rich plasma based on fluorometry and simple data processing.

Simultaneously, another revolutionary series of investigations were led by Mann and coworkers and a model for evaluating continuous thrombin generation in whole blood by meticulous subsampling and quantitation of thrombin production by enzyme-linked immunosorbent assay (ELISA) measurement of thrombin–antithrombin complexes was developed [1]. During the past decade, Mann *et al.* have also developed an *in silico*/computerized model of thrombin generation that can estimate the dynamic of thrombin generation with varied levels of coagulation factors and natural anticoagulants.

Throughout the past century investigations have demonstrated that changes in the dynamic- and rate-specific characteristics of thrombin generation and clot formation are associated with a variety of pathologic conditions ranging from hemophilia [4] leading to an increased bleeding tendency to the converse, thrombophilia leading to a predisposition to form pathologic thromboses [5]. Hence, measuring and characterizing thrombin generation has been the subject of intense and detailed research. This has led to an increasing number of laboratories having both the laboratory and intellectual capability to perform research utilizing thrombin generation measurement.

Calibrated automated continuous thrombin generation measurement

Hemker and Béguin have developed a simple principle of quantitating the dynamics of thrombin generation called the calibrated automated thrombin (CAT) generation method. The method is based on the use of plasma and a fluorogenic thrombin substrate as well as a 96-well plate fluorometer. Fluorescence measurements are associated with high degree of variability caused by nonlinearity of the fluorescence intensity. Furthermore, a fluorescence signal depends upon the quenching properties of the medium, and plasma samples differ widely—even samples of the same person obtained on the same day. Thus, in order to quantitate thrombin concentrations from the fluorescence signal, each sample has to be run in parallel with a calibrator (in the model described by Hemker, α-2-macroglobulin–thrombin complexes are used as calibrator). In practice, citrated platelet-poor plasma or platelet-rich plasma is added to the wells of the microplate. The fluorogenic thrombin substrate is then added and coagulation is activated by tissue factor. Following re-calcification, automated continuous measurements of fluorescence are started. A simple computer program converts the fluorescence readings into a quantitative dynamic profile of thrombin generation (Figure 40.1).

Measurement of thrombin generation by subsampling

The most sophisticated way of estimating thrombin generation is in fact also the most time-consuming, cumbersome, and

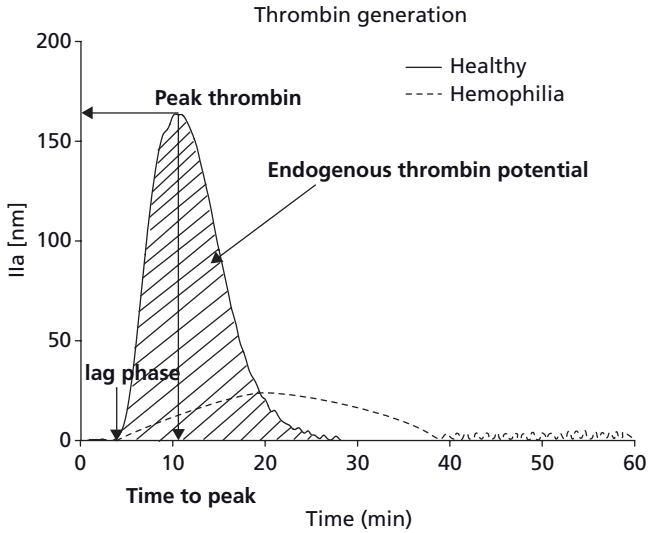

Figure 40.1 Thrombin generation. Characteristic thrombin generation profiles from a healthy individual and a patient with severe Hemophilia. As illustrated, the lag phase is not distinctly different between healthy and severe hemophilia; however, in hemophilia the propagation phase is compromised, as visualized by the low peak thrombin and reduced ETP.

complicated. Plasma or whole blood are distributed to multiple small test tubes. The test samples are re-calcified and the clotting process is triggered by an activator, most commonly tissue factor. At specific time intervals (e.g., 5 s), thrombin generation is stopped by addition of a cold quenching solution that terminates all enzymatic processes and stops thrombin generation. Subsequently, each sample is centrifuged and the supernatant is removed, and for each time point the total amount of thrombin is evaluated by ELISA-based measurement of thrombin–antithrombin complexes [1].

Laboratory facilities

Thrombin generation measurements are very sensitive. Hence, meticulous and systematic blood sampling procedures are very important. Moreover, centrifugation and pipetting of plasma needs to be performed in a careful and systematic manner. Calibrated automated thrombin generation measurement requires a qualified and trained laboratory technician. In particular, the subsampling method, according to Mann, requires unique laboratory skills. As of today, several types of fluorometers are available. The CAT method, as devised by Hemker, uses the Fluoroscan® fluorometer from Thermo Scientific. Other methodologic procedures have been described, using other types of fluorometers, various types of fluorogenic substrates, and various formulations and concentrations of tissue factor. So far, the laboratory equipment is not considered transportable.

Subject samples

Preferably, citrated blood samples should be taken using minimal stasis and a smooth vein puncture. Recently, citrate as a reversible anticoagulant has been shown to affect the dynamics of thrombin generation measurements [6]; however, as a practically useful alternative is not available, this is the preferred method for blood collection.

Many laboratories add corn trypsin inhibitor (CTI—a direct and potent inhibitor of coagulation factor XIIa) to the test tubes before blood sampling in order to avoid or minimize spontaneous contact activation. CTI has been demonstrated to reduce variability of the assay [7]. The thrombin generation analysis can be done using fresh or frozen platelet-poor (PPP) or fresh platelet-rich plasma (PRP). If PRP is used the analysis should be initiated with 120 min following blood sampling. The predominant activator of thrombin generation is tissue factor, and an increasing list of different tissue factor formulations and concentrations are available. The formulations and concentration of tissue factor have pronounced effects on the sensitivity and dynamic characteristics of the thrombin generation profiles. If thrombin generation is measured using a high concentration of tissue factor, it is difficult to visualize the dynamic abnormalities characteristic of hemophilia. The lower the concentration of tissue factor, the higher the sensitivity, but this is at the cost of increasing variability. The fluorogenic thrombin substrate needs to be slow reacting in order to hinder consumption. Several fluorogenic substrates are currently available.

Methods, interpretation, and clinical feasibility

The classic thrombin generation curve has a waveform from which several quantifiable parameters can be measured (lag time, time to peak thrombin, and endogenous thrombin potential as described below) (Figure 40.1). The entire course of thrombin generation is usually separated into an initiation phase corresponding the lag time before the earliest measurable thrombin. This is followed by the amplification phase, which likely represents the assembling of the intrinsic tenase (FIXa and FVIIIa) and the prothrombinase complex (FXa and FVa), during which the maximum rate of thrombin generation occurs. Eventually, thrombin generation reaches a peak and the time required to reach this point is described as time-to-peak thrombin. The total amount of thrombin generation (equal to area under the curve) is frequently called the endogeneous thrombin potential (ETP).

In principle, abnormal thrombin generation curves are characterized by (i) a prolonged or shortened lag time, (ii) reduced or increased peak thrombin, or (iii) reduced or elevated ETP.

The interpretation of the thrombin generation profile includes several variables. The lag time of thrombin is primarily determined by levels of free tissue factor, tissue factor pathway inhibitor, factor VII, factor IX, and fibrinogen [8]. The propagation phase of thrombin is highly dependent on the number and function of platelets [9]. Hence, the higher the platelet counts the higher the maximum rate and acceleration of thrombin generation. Other main determinants of the propagation phase of thrombin generation depend on the intensity of activation with tissue factor. Following activation with low levels of tissue factor, fibrinogen, factor XII, free tissue factor pathway inhibitor (TFPI), antithrombin, and prothrombin are important determinants of thrombin generation. Following stimulation with a high level of tissue factor, prothrombin, antithrombin, fibrinogen, free TFPI, and factor V become the predominant determinants of thrombin.

Males are described to have a lower thrombin production than females. In addition, thrombin generation increases with age and decreases as a result of low temperature.

Laboratory phenotyping of bleeding disorders and monitoring of hemostatic intervention

Thrombin generation has been used for laboratory phenotyping of a variety of bleeding disorders. The best characterized hemostatic dysfunction described by thrombin generation profiles is hemophilia A. In contrast to the categoric distinction between mild, moderate, and severe based on assessment of functional levels of factor VIII, the rate-specific characteristics of thrombin generation have been reported to illustrate and reflect the clinical heterogeneity of hemophilia [10]. In

particular, thrombin generation has been documented as predictive in distinguishing milder phenotypes of severe hemophilia despite similar low levels of factor VIII (e.g., less than 1% of normal). Noteworthy, so far the predictive value of thrombin generation has only been significant when using PRP as a test medium.

Thrombin generation profiles have also been used for monitoring the hemostatic response to various types of hemostatic treatment [11,12]. Severe hemophilia is usually managed by substitution with a factor VIII concentrate. A considerable proportion of patients develop allogeneic inhibitory antibodies toward the substitution therapy. These patients require so-called bypassing agents, such as recombinant factor VIIa or plasma-derived activated prothrombin complex concentrates. Thrombin generation measurement has been used to monitor substitution with such bypassing agents. The overall experience with thrombin measurements as a surrogate parameter of hemostatic effect is still rather limited; however, the preliminary results appears promising.

Outside hemophilia, thrombin generation has proven advantageous for illustrating the hemostatic potential of, for example, prothrombin complex concentrates as compared with fresh-frozen plasma for reversal of vitamin K antagonist therapy [13].

Finally, thrombin generation is an elegant method for illustrating the impact of various types of anticoagulation. Heparin, direct thrombin inhibitors, as well as indirect or direct factor Xa inhibitors compromise thrombin generation by prolonging the lag phase and reducing peak thrombin and ETP.

Recording of whole blood clot formation

History

Throughout history investigators of blood coagulation have made great efforts to establish ways for monitoring the hemostatic capacity. A pioneer test was the whole blood clotting time, reported in 1780 by Hewson, who measured clotting times in basins, describing the duration of time until visible clotting was seen in freshly drawn whole blood [14]. The principle of a whole blood coagulation test is the measurement of physical changes occurring during clot formation. Instrumented whole blood clotting time studies were introduced in 1910 by Kottmann, who continuously measured the increment of viscosity during clotting by his "Koaguloviskosimeter" [14] and later, in 1948, Hartert introduced thromboelastography [15]. This enabled analysis of continued clot formation in whole blood as well as in plasma. The thromboelastograph of Hartert was based on a cup mounted in a cuvette that oscillated four degrees in opposite directions at a frequency of 3.5 s [15]. Inserted in the center of the cup was a pin attached to a torsion wire. The resistance of the blood was kymographically recorded as a consequence of increased movement of the pin: "*r*" was the reaction time and "*k*" ("k" for the German word "klot") was the period in which the blood's "elasticity" reached half of its eventual peak value, "*m*". Thus, continued clot formation was demonstrable and visualized.

There are now several different methods and technologies for recording and visualizing continuous whole blood clot formation. In the area of hemophilia, predominantly thromboelastography has been investigated; however, other methods may emerge also.

Instrumentation and materials

The thromboelastographic principle introduced by Hartert has been adopted in a computerized version of the TEG® apparatus manufactured by Haemoscope. In 1996 Calatzis *et al*. invented another principle of thrombelastography, today named thromboelastometry (ROTEM®) in which the pin, instead of the cup, oscillates [16]. A ball bearing focusing the pin apparently makes the ROTEM less sensible for movements.

Both the TEG and the ROTEM provide a digital signal allowing for additional computation of the continuous coagulation signal leading to the derivation of several quantifiable parameters (Figure 40.2a and b).

The underlying principle of the TEG and ROTEM is the continuous assessment of the elastic properties of a forming clot. Both devices consist of a cup into which the sample (whole blood, platelet-rich or -poor plasma) and reagents are placed, and of a pin which sits in the center of the cup when the device is running. Once the sample is in place and the cup is pushed up into the pin, the pin (ROTEM) or cup (TEG) begins to oscillate. Reduced movement of the pin during clot formation is registered with specialized computer software and visualized on a computer, providing a coagulation signal similar to that of the traditional thromboelastography (Figure 40.2a). The formation of strands of fibrin which attach the pin to the wall of the cup create this effect.

Laboratory facilities

TEG and ROTEM were originally developed and validated as bedside monitoring tools for diagnosis of perioperative coagulopathies and guidance of optimal hemostatic intervention as they are easy to use and transportable. Several commercial standard assays are available, providing activation of the intrinsic pathway (e.g., with kaolin) or extrinsic pathway (with tissue factor) as well as fibrinogen-sensitive assays and assays to neutralize heparin.

In addition to the standard assays, a series of studies have used an in-house developed assay employing activation with minute amounts of tissue factor (dilution of 1:17 000 to 1:50 000 of standard concentrations for performing a PT) [17,18]. The low tissue factor assays provide a high sensitivity; however, they are also associated with increased variability. The variability can be reduced significantly by prespiking citrated blood tubes with CTI.

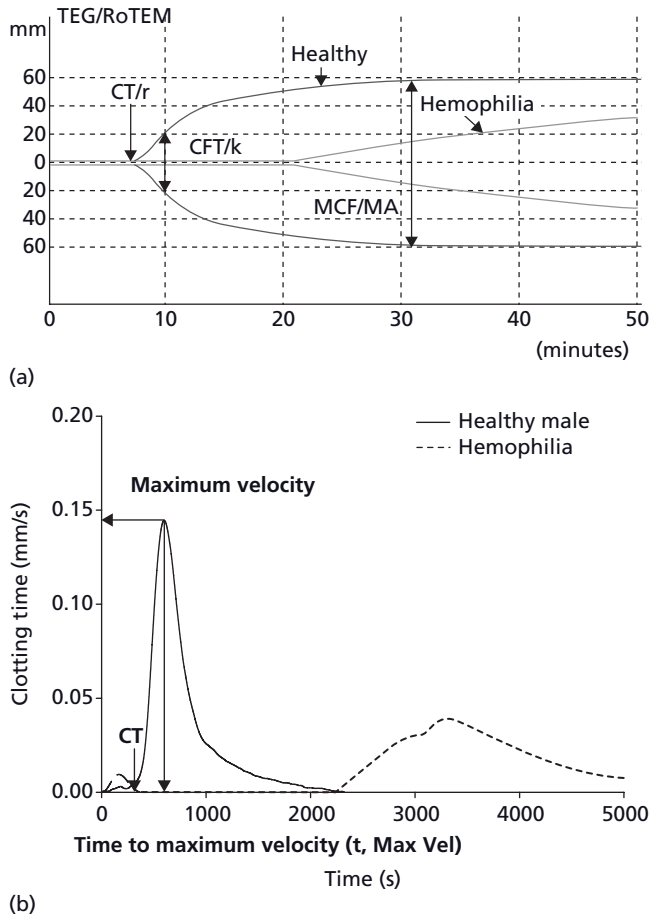

Figure 40.2 TEG®/RoTEM®. (a) Characteristic thromboelastographic profiles from a healthy individual as well as a patient with severe Hemophilia. (b) The first derivative of the thromboelastographic prfile. As illustrated, the clotting time is prolonged and the maximum velocity is reduced in Hemophilia. Fulthermore, after 50 minutes, the Hemophilia sample has not reached a maximum clot firmness similar to the healthy sample.

Subject samples

Although the devices were developed as whole blood assays, essentially any coagulable liquid can be placed in the device, including platelet-rich and -poor plasma. Samples should be drawn from an atraumatic, fresh venipuncture with a free flow of blood. It is suggested that the first 2–3 ml be discarded because of potential clotting activation. With respect to whole blood, either native whole blood or recalcified, anticoagulated (citrated) blood can be used. While native whole blood has been used in some settings (the operating room, for example), it is not very practical as the blood needs to be placed in the device within 4 min. It is best to utilize blood anticoagulated with –3.2% sodium citrate at a 9:1 ratio. A recent study comparing native blood with citrated blood showed no difference in the assay results. It is suggested that samples should be run within 2 h, although studies of the duration of time from sampling to running the assay have not been performed.

Methods, interpretation, and clinical feasibility

The classic thromboelastographic profile is illustrated in Figure 40.2. It illustrates the continuous viscoelastic physical changes occurring during whole blood coagulation. Traditional thromboelastographic parameters such as clotting time (*r*, CT), clot formation time (*k*, CFT), and maximal clot formation (MA, MCF) are depicted in Figure 40.2. *r*/CT expresses a measure of the initiation of clot formation. In addition to the above parameters, the device can assess the degree of fibrinolysis by demonstrating a diminution of MA/MCF following attainment of a plateau (Figure 40.2). This is assessed quantitatively by the LY30 or LY60, which is the lysis index at 30 or 60 min and acts as a measure of the decrease of MA.

Furthermore, the digital raw signal from the TEG or ROTEM analyzers can be differentiated to velocity profiles of whole-blood clot formation and dynamic coagulation parameters illustrating the propagation phase of clotting can be derived (Figure 40.2b) such as the maximum velocity (MaxVel) of clot formation and the time until the occurrence of the maximum value (*t*, MaxVel) [17].

Tissue factor activation methods

As tissue factor is the physiologic activator of coagulation, it is thought that utilizing tissue factor in thromboelastography more closely mimics the natural state and will thus yield more meaningful results. In a series of studies of thromboelastography, recombinant human tissue factor has been chosen as the tissue factor source. So far, studies have demonstrated that using minute amounts of tissue factor (e.g., final dilution of Innovin® ranging from 1:17 000 to 1:50 000) can demonstrate differences between normal subjects and patients with varying degrees of hemophilia [17,19]. Furthermore, additional studies demonstrated that *ex vivo* addition of factor or bypassing agents could be detected [17,20–24]. More recently, it has been shown that the addition of CTI improves the performance of the assay when utilizing tissue factor activation [23].

Contact pathway activation methods

Activation of the intrinsic system, while not physiologic, has been used for decades in a wide variety of clotting assays. In the setting of hemophilia, contact activation (with, for example, kaolin or synthasil) has been utilized successfully in an *in vivo* study of dose individualization in inhibitor patients, as well as to monitor dosing of bypassing agents during major orthopedic surgery [25]. The kaolin method is simple as the manufacturer provides prefilled kaolin vials and dilution is not required.

In principle, abnormal thromboelastographic/thromboelastometry profiles are characterized by (i) a prolonged or shortened *r* or CT, (ii) prolonged *k* or CFT, or (iii) a compromised or elevated MA or MCF.

Laboratory phenotyping of bleeding disorders and monitor of hemostatic intervention

With respect to prediction of the clinical phenotype, a number of studies have demonstrated considerable heterogeneity in the baseline whole blood coagulation patterns among patients with verified factor VIII levels < 1% [19]. Furthermore, data have illustrated that patients diagnosed with severe hemophilia A (FVIII:C < 1%) but having unusually good whole blood clotting profiles are associated with a less severe bleeding phenotype [22]. The low tissue factor assay has also been used to illustrate different response patterns to various levels of coagulation factor VIII concentrate [19]. In addition, both *in vitro* and *in vivo* studies have demonstrated the ability of thromboelastography to predict the clinical response to bypassing agents in patients with inhibitors [17,18,20,21,26]. So far, these studies represent single-center experiences. Nevertheless, the potential for thromboelastography has been demonstrated. An *in vivo* study demonstrated the ability of thromboelastography to individualize therapy for three inhibitor patients, which led to more judicious use of bypassing agents as well as more convenient treatment regimens, ultimately reducing the cost of managing bleeds without compromising efficacy (unpublished). Additional studies are required in order to answer some of the questions raised by these initial studies, including which preanalytic methods provide the most sensitive, reliable, and reproducible results. In the near future, it is possible that thromboelastography may be part of the tools used to design individual treatment regimens for patients with inhibitors. This will become increasingly important as new bypassing agents become available.

References

1 Mann KG. Biochemistry and physiology of blood coagulation. *Thromb Haemost* 1999; **82**: 165–74.

2 Brummel KE, Paradis SG, Butenas S, Mann KG. Thrombin functions during tissue factor-induced blood coagulation. *Blood* 2002; **100**: 148–52.

3 Hemker HC. Recollections on thrombin generation. *J Thromb Haemost* 2008; **6**: 219–26.

4 Hemker HC, Beguin S. Phenotyping the clotting system. *Thromb Haemost* 2000; **84**: 747–51.

5 Brummel-Ziedins KE, Vossen CY, Butenas S, Mann KG, Rosendaal FR. Thrombin generation profiles in deep venous thrombosis. *J Thromb Haemost* 2005; **3**: 2497–505.

6 Mann KG, Whelihan MF, Butenas S, Orfeo T. Citrate anticoagulation and the dynamics of thrombin generation. *J Thromb Haemost* 2007; **5**: 2055–61.

7 van Veen JJ, Gatt A, Cooper PC, Kitchen S, Bowyer AE, Makris M. Corn trypsin inhibitor in fluorogenic thrombin-generation measurements is only necessary at low tissue factor concentrations and influences the relationship between factor VIII coagulant activity and thrombogram parameters. *Blood Coagul Fibrinolysis* 2008; **19**: 183–9.

8 Dielis AW, Castoldi E, Spronk HM, *et al.* Coagulation factors and the protein C system as determinants of thrombin generation in a normal population. *J Thromb Haemost* 2008; **6**: 125–31.

9 Kjalke M, Ezban M, Monroe DM, Hoffman M, Roberts HR, Hedner U. High-dose factor VIIa increases initial thrombin generation and mediates faster platelet activation in thrombocytopenia-like conditions in a cell-based model system. *Br J Haematol* 2001; **114**: 114–20.

10 Dargaud Y, Beguin S, Lienhart A, *et al.* Evaluation of thrombin generating capacity in plasma from patients with haemophilia A and B. *Thromb Haemost* 2005; **93**: 475–80.

11 Dargaud Y, Bordet JC, Lienhart A, Negrier C. Use of the thrombin generation test to evaluate response to treatment with recombinant activated factor VII. *Semin Hematol* 2008; **45**(Suppl. 1): S72–S73.

12 Dargaud Y, Lienhart A, Meunier S, *et al.* Major surgery in a severe haemophilia A patient with high titre inhibitor: use of the thrombin generation test in the therapeutic decision. *Haemophilia* 2005; **11**: 552–8.

13 Gatt A, van Veen JJ, Woolley AM, Kitchen S, Cooper P, Makris M. Thrombin generation assays are superior to traditional tests in assessing anticoagulation reversal *in vitro*. *Thromb Haemost* 2008; **100**: 350–5.

14 Owen CA. *The History of Blood Coagulation*, 1st edn. Rochester: Mayo Foundation for Medical Education and Research, 2001.

15 Hartert H. Blutgerinnungsstudien mit der thrombelastographie, einem neuen untersuchungsverfahren. *Klinische Wochenschrift* 1948; **26**: 577–83.

16 Calatzis A, Fritzsche P, Calatzis A, Kling M, Hipp R, Stemberger A. A comparison of the technical principle of the roTEG coagulation analyser and conventional thromboelastographic systems. *Ann Haematol* 1996; **72**(Suppl. 1): P90 (abstract).

17 Sørensen B, Johansen P, Christiansen K, Wöelke M, Ingerslev J. Whole blood coagulation thromboelastographic profiles employing minimal tissue factor activation. *J Thromb Haemost* 2003; **1**: 551–8.

18 Sørensen B, Persson E, Ingerslev J. Factor VIIa analogue (V158D/E296V/M298Q-FVIIa) normalises clot formation in whole blood from patients with severe haemophilia A. *Br J Haematol* 2007; **137**: 158–65.

19 Ingerslev J, Poulsen LH, Sørensen B. Potential role of the dynamic properties of whole blood coagulation in assessment of dosage requirements in haemophilia. *Haemophilia* 2003; **9**: 348–52.

20 Sørensen B, Ingerslev J. Whole blood clot formation phenotypes in hemophilia A and rare coagulation disorders. Patterns of response to recombinant factor VIIa. *J Thromb Haemost* 2004; **2**: 102–10.

21 Sørensen B, Ingerslev J. Tailoring haemostatic treatment to patient requirements—an update on monitoring haemostatic response using thrombelastography. *Haemophilia* 2005; **11**(Suppl. 1): 1–6.

22 Chitlur M, Warrier I, Rajpurkar M, *et al.* Thromboelastography in children with coagulation factor deficiencies. *Br J Haematol* 2008; **142**: 250–6.

23 Rivard GE, Brummel-Ziedins KE, Mann KG, Fan L, Hofer A, Cohen E. Evaluation of the profile of thrombin generation during the process of whole blood clotting as assessed by thrombelastography. *J Thromb Haemost* 2005; **3**: 2039–43.

24 Young G, Blain R, Nakagawa P, Nugent DJ. Individualization of bypassing agent treatment for haemophilic patients with inhibitors utilizing thromboelastography. *Haemophilia* 2006; **12**: 598–604.

25 Ingerslev J, Christiansen K, Calatzis A, Holm M, Sabroe EL. Management and monitoring of recombinant activated factor VII. *Blood Coagul Fibrinolysis* 2000; **11**(Suppl. 1): S25–S30.

26 Sørensen B, Ingerslev J. Thromboelastography and recombinant factor VIIa—hemophilia and beyond. *Semin Hematol* 2004; **41**(Suppl. 1): 140–4.

41 Obstetrics and gynecology: hemophilia

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Introduction

Hemophilia A and B are X-linked recessive bleeding disorders caused by deficiency of factor VIII (FVIII) or factor IX (FIX), respectively. Because of the mode of inheritance, hemophilia A and B mostly affect males, while females are carriers. Therefore, the clotting factor level is expected to be around 50% of normal in carriers as they have only one affected chromosome. However, a wide range of values (22–116 IU/dL) has been reported [1] as a result of random inactivation of one of the two X chromosomes, the process called lyonization [2]. A significant number of hemophilia carriers may have very low factor levels due to extreme lyonization, thus at an increased risk of bleeding. Carriers of hemophilia with mildly reduced clotting factor levels (40–60 IU/dL) are also at risk of bleeding, especially after medical intervention [3]. Women are exposed to regular hemostatic challenges owing to monthly menstruation as well as childbirth, therefore at risk of menorrhagia and postpartum hemorrhage.

Management of obstetric and gynecologic problems in carriers of hemophilia requires a multidisciplinary approach and close collaboration between gynecologists and the hemophilia center. Ideally, these women should be managed in a combined clinic, where expertise and facilities are available to provide a comprehensive assessment and management plan for their problems [4]. Advice from the hemophilia team is invaluable for arrangement and interpretation of blood tests and arrangement of prophylactic or replacement treatment, especially when there is a bleeding complication.

Gynecology

Menorrhagia, heavy menstrual loss, is a common gynecologic problem among women with bleeding disorders, including carriers of hemophilia. In a study using a pictorial blood assessment chart (PBAC), menstrual loss was assessed in 30 carriers of hemophilia; the median menstrual score was 113, significantly higher than 73 in the age-matched control group. The incidence of menorrhagia, as defined by a score of more than 100, was 57% compared with 29% in the control group [5]. In addition to heavy menstruation, carriers of hemophilia may also suffer prolonged menstruation with episodes of flooding and passage of clots [5]. Lack of awareness of the high incidence of menorrhagia and underestimation of menstrual loss in these women may lead to chronic iron-deficiency anemia as well as inappropriate management and unnecessary surgical intervention, including hysterectomy, at early reproductive age [5]. Carriers of hemophilia also suffer a significant menstrual pain, intermenstrual bleedings, and mid-cycle pain. Mid-cycle pain probably arises from ovulation with subsequent hemorrhage into the corpus luteum or peritoneal irritation because of bleeding from the edges of a recently formed corpus luteum. Acute abdomen owing to hemoperitonium, as well as extension of bleeding into the broad ligament with spontaneous rupture of corpus luteum, has been reported in carriers of hemophilia [6]. Although this is a rare complication, it is important to be considered in these patients before embarking on any surgical intervention, as conservative management with factor replacement is usually effective and obviates the need for surgery.

Menorrhagia and dysmenorrhea adversely affect quality of life of women and may have a major influence on women's lifestyle and employment. Carriers of hemophilia, especially those who passed clots, suffered flooding and had prolonged menstruation, have been shown to have a significantly worse quality of life during menstruation compared with a control group [7]. Between 39% and 46% of them lost time from work [7,8]. They also accomplished less at work and experienced difficulties performing their work. Like other bleeding symptoms, presence and severity of menorrhagia correlate with the carrier's factor level. Carriers with factor levels below 40 IU/dL are more likely to have menorrhagia and suffer form iron-deficiency anemia and adverse quality of life [3].

Management of menorrhagia

Menorrhagia in carriers of hemophilia is most likely to be because of their clotting factor deficiency, but not exclusively. Therefore, each individual should be appropriately assessed and local causes excluded, especially the possibility of malignancy in older women. The treatment of menorrhagia is

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usually medical and the most commonly used first-line options are tranexamic acid, combined oral contraceptive pills, or desmopressin (DDAVP, self-administered by subcutaneous injection or as intranasal spray). The choice is dependent on the patient's age, reproductive state, and preference and availability of the medications, as well as the clinician's experience and preference. The levonorgestrel intrauterine system, Mirena (LNG-IUS), has also been shown to be highly effective in reducing menstrual loss and well tolerated in women with bleeding disorders [9,10]. It is the treatment of choice for women who also require contraception. The main problem with LNG-IUS is a high discontinuation rate because of irregular bleeding/spotting during the first 3–6 months and the progestogenic side-effects. Proper counseling and patient education may increase tolerance. In women with bleeding disorders, this option should be considered prior to any surgical management.

Surgical intervention is sometimes required in patients unresponsive to medical treatment. Surgical procedures, even relatively minor operations, can be complicated by hemorrhage in some carriers of hemophilia. Therefore, good liaison between the local hemophilia center and the surgical/anesthetic team is essential. An adequate hemostatic cover should be provided with the aim of maintaining the clotting factors >50 IU/dL until healing is complete. The treatment may need to be continued post operatively, sometimes for up to 10 days, to reduce the development of secondary bleeding and hematomas. Surgery should be performed by expert gynecologists, choosing a technique with the least risk of bleeding complication.

Genetics

Genetic counseling and carrier detection

The purpose of genetic counseling is to provide the potential carrier and her parents or partner with adequate information to reach a decision regarding carrier testing and prenatal diagnosis and to provide support throughout the process [11]. Genetic counseling requires an empathetic, good communicator who has a detailed knowledge of hemophilia, genetics, molecular biology, and prenatal diagnostic procedures. Counseling is a way of addressing the implication of the information that is given. It is a two-way process, enabling a better understanding between the patient and healthcare worker about the full range of issues. Genetic counseling in hemophilia focuses on the medical condition (what it is, how it is treated, and how it is passed from generation to generation), personal and relationship concerns related to hemophilia, and beliefs and wishes about the person discussing possible inheritance, as well as those who might be affected.

Ethical considerations include human rights, issues surrounding consent, and those relating to confidentiality. Sometimes the best interests of the person with hemophilia and the partner, sister, or child of a carrier can be in conflict—genetic counseling needs to address and consider these issues.

Table 41.1 Counseling and consent for genetic testing.

Establish that hemophilia is present in the family and determine its type and severity
Establish family pedigree (tree) to identify possible or definite (obligate) carriers
Provide a full explanation of the potential clinical effects of being a hemophilia carrier or affected male
Provide a full explanation of mode of inheritance of hemophilia
Discuss the rationale for identifying the genetic defect in patients with hemophilia
Outline the means by which carrier status is assessed
Discuss what is involved in genetic testing: sample collection, transfer/storage of data, research projects on stored material, insurance issues and risk of error
If appropriate, advise on the techniques for prenatal testing
Provide an opportunity to ask questions
Provide an opportunity for the individual being consulted to present her understanding of the information that has been discussed
Provide patient information sheet and an opportunity for follow-up appointment

A framework for genetic service provision has been provided as a guideline by the UK Haemophilia Centre Doctors' Organisation (UKHCDO) [12], and this includes detailed guidance for carrier diagnosis and antenatal diagnosis, which is summarized in Table 41.1.

The pedigree or family tree is the first step in carrier diagnosis. The daughter of a man with hemophilia is an obligate carrier and thus her sons have a 50% chance of having hemophilia and her daughters have a 50% chance of being a carrier. Within a family, where an index patient with hemophilia has been identified, many of the females may be at risk of being carriers. However, in many countries, more than 50% of newly diagnosed cases are sporadic [13]. In the mother of a sporadic case of hemophilia there is the possibility of mosaicism, a mixture of normal and mutation-carrying cells. It is important to establish maternal carriership before pregnancy and certainly before embarking on prenatal diagnosis.

Testing the carrier status of healthy children

The testing of the carrier status of healthy children for recessively inherited conditions has recently been reviewed [14]. There are ethical issues about who can give consent and what is the earliest age testing should be offered.

In a statement by the United Nations Convention on the rights of the child, it was held that any action or decision affecting this group should be in his or her "best interests" [15].

The World Health Organization (WHO) has proposed the reason for genetically testing children: to improve their medical care rather than to obtain reproductively significant informa-

tion [16]. A more flexible approach suggests that when thinking about the carrier testing of children, the health professional should recognize the dynamics and cohesion within the family [17]. A recent systematic review of guidelines and position papers has found an overall agreement in the recommendation to wait until children can give informed consent, but there were some exceptions [18].

One of the few papers to consider the age of testing by the hemophilia community itself was a survey on "the attitudes towards and beliefs about genetic testing in the haemophilia community" [14]. Interviews with 39 individuals including men with hemophilia, female carriers, and family members found that most thought testing was necessary for adolescent girls to determine carrier status to help prepare families for a child with hemophilia, rather than leading them to choose to terminate a pregnancy or not to have children.

Carrier diagnosis may be suggested by a low level of factor VIII (FVIII) or FIX. Until molecular diagnosis became possible, the mainstay of laboratory diagnosis of carriers of hemophilia A was the ratio of FVIII activity to factor VIII-related antigen. This may be the only method available in less resourced countries [1]. It has been shown that women with a clotting factor level of 40 IU/dL have three times increased risk of prolonged bleeding after operation compared with a clotting factor level of 60 IU/dL or above [3]. It is therefore recommended that the respective clotting factor is measured in young obligate carriers [12]. The ethical debate is redundant in these situations.

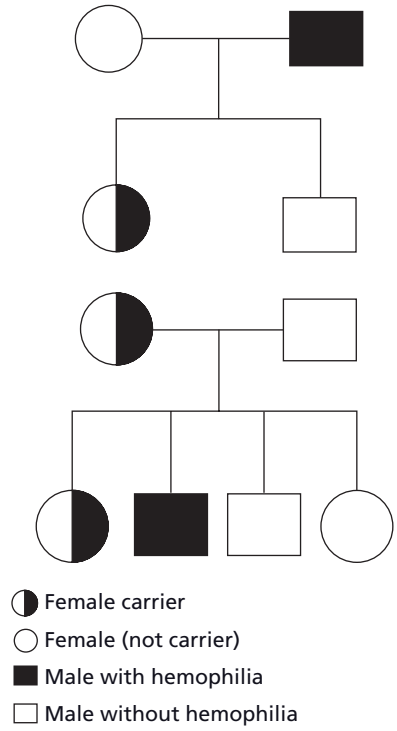

Figure 41.1 Inheritance of hemophilia.

Genetic diagnosis

It is now possible to perform genetic diagnosis. In severe hemophilia A approximately 50% of families carry an inversion of intron 22—a rearrangement of the long arm of the X chromosome [19]—and thus it may be possible to confirm carriership without knowledge of the mutation in the index patient within a family. However, for most potential carriers it is necessary to know the mutation in the index family member. Such mutations are listed on the HAMSTeRS database (http://europium.csc.mrc.ac.uk) for hemophilia A. In hemophilia B, almost every family has a unique mutation and in the UK most of these have been identified [20].

Prenatal diagnosis

Prenatal diagnosis (PND) forms an integral part of the care provided for carriers of hemophilia and their families. In each pregnancy, carriers of hemophilia have a 50% chance of having a male fetus that is affected and a 50% chance of having a female fetus that is also a carrier (Figure 41.1). Decisions made regarding the uptake of PND and termination of pregnancy are complex and influenced by multiple factors [21]. The majority of carriers of hemophilia do not consider hemophilia to be a sufficiently serious disorder to justify termination of pregnancy [22]. Therefore, their uptake for PND and termination of affected pregnancies has been reported to be generally low in several series [23]. However, the attitudes toward these procedures vary widely among countries, religions, and cultures, and over time. Economic factors may also influence the use of these techniques. The severity of hemophilia in the family and having a family member who experienced a tragic complication, such human immunodeficiency virus (HIV)/hepatitis C infection or inhibitor problems also influences decision-making regarding PND and termination of pregnancy [24].

Women may also choose not to have invasive testing because of the associated risk of miscarriage. In the last decade, there have been several advances in the PND of hemophilia. Determination of fetal sex is now possible in the first trimester through analysis of free fetal DNA in maternal circulation or by ultrasound assessment of the fetal genital tubercle [25]. This has been shown to be a reliable and useful method for avoiding invasive prenatal diagnostic tests in female pregnancies. In a recent series, the uptake for noninvasive fetal sex determination was high (71%, 46/65 pregnancies) whilst the uptake for invasive testing (chorionic villus biopsy) remained low (20%, 13/65 pregnancies) [23].

Chorionic villus biopsy (CVS) is the most widely used method for PND of hemophilia. It offers the advantage of attaining an early diagnosis and thus a shorter period of uncertainty compared with amniocentesis. Early diagnosis is

of particular importance when selective termination of affected pregnancy is being considered, as there may be personal or religious prohibitions on late termination of pregnancy. Furthermore, termination at a later gestation is more likely to be traumatic and associated with maternal complications. CVS is usually performed between 11 and 14 weeks of gestation under direct ultrasound guidance to obtain a sample of chorionic villi (placenta) for genetic analysis. Fetal DNA is extracted from the chorionic villus tissue and used for polymerase chain reaction (PCR)-based testing to determine fetal gender and direct mutation detection or polymorphism linkage analysis if the fetus is male. Results are usually available within 48–72 h of receipt of samples. CVS is associated with 1–2% risk of pregnancy loss [26]. It is recommended that CVS should not be performed before 10 weeks of gestation because of its association with limb reduction deformities when carried out before this gestation [27,28]. There is a small (<1%) chance of failing to obtain a result from the laboratory test. Amniocentesis can also be used for PND. This technique is generally performed between 15 and 18 gestational weeks. Amniotic fluid contains fetal cells from which rapid detection of the sex chromosomes can be achieved by fluorescence *in situ* hybridization (FISH). DNA can also be extracted directly and used for PCR-based testing for linkage analysis or direct mutation detection, the results of which are usually available within 48–72 h. However, there is often insufficient DNA present in the sample for analysis. Therefore, the testing may be delayed until cultured cells are available, which takes approximately 2 weeks. The risk of miscarriage is 0.5–1% [26,29]. Cordocentesis, or percutaneous umbilical cord blood sampling, to assess fetal clotting factors is another invasive diagnostic technique performed after 18 weeks of gestation. Cordocentesis has now largely been superseded by CVS and amniocentesis in obtaining fetal material for genetic studies. The latter two procedures allow earlier diagnosis, are less technically demanding, and are associated with a lower risk of complications. Cordocentesis is only considered when the causative mutation in the family cannot be determined. It carries an approximately 1–2% risk of procedure-related fetal loss [30]. There is a risk of transient cord hematoma or bleeding from the puncture site, but could potentially be significant in affected fetuses.

Noninvasive fetal gender determination in the first trimester is valuable for prenatal diagnosis of hemophilia as it allows the avoidance of invasive testing in female pregnancies [25]. Fetal gender can now be determined at 11–14 weeks by ultrasound assessment of the fetal genital tubercle [25,31]. Accuracy of this method increases with advancing gestation from 70.3% at 11 weeks to 98.7% at 12 weeks and 100% at 13 weeks [31]. Another noninvasive method of determining fetal gender is the analysis of free fetal DNA in maternal circulation. Using real-time quantitative PCR, several groups have demonstrated a 100% sensitivity and specificity in the detection of male fetuses [25,32,33]. The combined use of both methods increases the confidence of patients and clinicians in the accuracy of these tests and provides carriers of hemophilia with a reliable option of avoiding invasive testing in female pregnancies [25]. Knowledge of fetal sex is also helpful for management of delivery in carriers who have not undergone specific PND. In these circumstances, the risk of traumatic hemorrhage can be minimized by avoiding the invasive monitoring techniques and instrumental deliveries in male fetuses (see Management of labor and delivery).

Management of pregnancy

Serum FVIII level increases progressively in carriers of hemophilia A during pregnancy, reaching its peak in the third trimester. Consequently, most carriers of hemophilia A have normal (>50 IU/dL) FVIII levels at term [23,34]. In contrast, FIX levels do show significant change with gestation and most carriers of hemophilia B with a low baseline (nonpregnant) level will continue to have the hemostatic defect at term (Figure 41.2) [23,34].

At present, there is insufficient evidence to determine whether the risks of miscarriage and antepartum bleeding are increased among carriers of hemophilia. The efficacy and need for antenatal prophylaxis among those with low (<50 IU/dL) factor levels are also debatable. However, prophylactic treatment is recommended to cover invasive procedures in carriers with factor levels below 50 IU/dL to prevent bleeding complications. Prophylactic treatments are commonly used for invasive procedures (such as prenatal tests, termination of pregnancy, etc.) in the first and second trimester even in carriers of hemophilia A, as the rise in FVIII level may only become significant in late gestation. In the UK series [23], prophylaxis was used in 59% (10/17) of carriers to cover invasive prenatal diagnostic procedures because the mother's factor levels were < 50 IU/dL.

Ideally, clotting factor levels should be checked at booking, and at 28 and 34 weeks of gestation, especially in those with low prepregnancy levels. Assessing factor levels at planned intervals allows their availability in acute situations when factor levels often cannot be assessed easily. Monitoring in the third trimester is of particular importance for appropriate management of labor and delivery.

Management of labor and delivery

Carriers of hemophilia and their unborn babies may be exposed to various hemostatic challenges during the process of labor and delivery, therefore potentially at risk of bleeding complications. Therefore, close liaison with the hemophilia center/hematologists throughout the pregnancy is essential and arrangement and plan for delivery should be made in advance. Delivery at a tertiary obstetric unit with an on-site hemophilia center is not required for all carriers of hemophilia. However, it is recommended that carriers with low

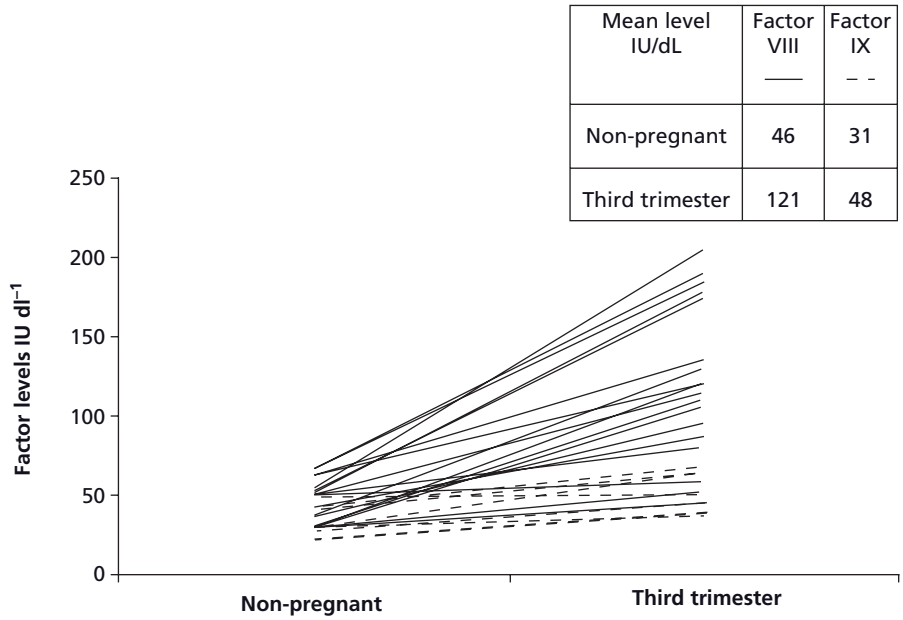

Figure 41.2 Changes in factor VIII and factor IX during pregnancy.

factor levels at term and those carrying an affected/potentially affected male fetus to deliver at a unit where the necessary expertise in the management of bleeding disorders and resources for laboratory testing and clotting factor treatments are readily available [35]. In these circumstances, planned delivery may be considered to ensure timely arrival of the mother to the tertiary center during labor [36]. However, induced labor is more likely to be prolonged and associated with the need for instrumental delivery or emergency cesarean section. Therefore, spontaneous delivery should be allowed whenever possible. In certain situations when induction of labor is expected to be prolonged, an elective cesarean section could be considered less traumatic, especially if the fetus is affected with hemophilia.

The fetus

Affected fetuses are potentially at risk of bleeding complication during labor and delivery, including the risk of serious cranial bleeding (cephalohematoma and intracranial hemorrhage) from the process of birth. The safest method of delivery for fetuses at risk is controversial. In a survey of obstetricians in the USA, 57% would "frequently" deliver a known carrier of hemophilia by vaginal route, while 11% preferred a cesarean section; 85% and 74% responded would rarely use vacuum extraction and forceps delivery, respectively [37]. Cranial bleeding, both intracranial and extracranial hemorrhage (ICH and ECH), is the most common site of bleeding in newborns with hemophilia [38]. In addition, ICH has the highest potential of all sites of bleeding for long-term serious sequelae such as seizures and neurologic impairment [39]. Neonates with severe hemophilia are at the highest risk, but it can occur even in newborns with mild or moderate bleeding disorders. Cranial hemorrhages in newborns with hemophilia occurred in 59%, 31%, and 10% of severe, moderate, and mild hemophilia, respectively [40]. The risk factors for cranial bleeding during labor and delivery include prolonged labor, especially during the second stage of labor, and instrumental deliveries particularly vacuum extraction.

Ljung *et al*. [41] reviewed 117 children with moderate to severe hemophilia born in Sweden between 1970 and 1990 and found 23 neonatal bleedings associated with delivery, including 16 cranial bleedings. The risk of cranial bleeding was 3% (3/87) with vaginal delivery, 64% (11/17) with vacuum extraction, and 15% (2/13) with cesarean section. In a UK study, cephalohematomas were also reported after a ventouse delivery and an emergency cesarean section after a prolonged second stage of labor [34]. Based on these data, cesarean section is not expected to eliminate the risk of bleeding complications in affected neonates and hence cannot be recommended in all pregnancies at risk of hemophilia [23,34,41]. Normal vaginal delivery is generally not contraindicated; however, the use of vacuum extraction or difficult forceps and prolonged labor, especially prolonged second stage of labor, should be avoided since they are associated with an increased risk of cephalohematoma or intracranial bleeding [35,36,41]. In principle, delivery should be achieved by the least traumatic method and early recourse to cesarean section should be considered to minimize the risk of neonatal bleeding complications. Low forceps delivery may be considered less traumatic than cesarean section when the head is deeply engaged in the pelvis and an easy outlet delivery is anticipated. Invasive intrapartum monitoring techniques such as fetal scalp electrodes and fetal blood sampling should also be avoided owing to the potential risk of scalp hemorrhage.

Knowledge of fetal sex is very useful for management of labor in carriers of hemophilia. If the fetus is identified as male, there is a 50% chance of being affected. If specific prenatal diagnosis has not been performed, it is important to treat the pregnancy as potentially affected and avoid invasive intrapartum monitoring and instrumental deliveries. If the fetus is identified as female, there is a 50% chance of being a carrier like her mother. There are no specific data on the bleeding risks of newborns who are carriers of hemophilia, but they are usually not expected to be at increased risk of bleeding complications. Furthermore, recent data suggest that the process of labor leads to a significant increase in neonatal factor levels [42]. However, some carriers could have significantly low factor levels owing to extreme lyonization [2]. Therefore, invasive techniques that carry a significant risk of head bleeding, such as vacuum extraction, should preferably be avoided in these cases.

After delivery, a cord blood sample should be collected to assess coagulation status and factor levels. When assessing the neonatal factor levels, it should be appreciated that these correlate with gestational age and reach adult levels at 6 months of age. It is therefore not reliable to diagnose mild forms especially in case of hemophilia B. If delivery has been traumatic or if there are clinical signs suggestive of head bleeding, a cranial ultrasound should be performed. It is also advisable to consider prophylactic cover in these cases [35]. Intramuscular injections should be avoided, vitamin K should be given orally and routine immunizations given intradermally or subcutaneously.

The mother

Carriers of hemophilia are at an increased risk of bleeding during labor, delivery, and postpartum period, especially those with factor levels below 50 IU/dL [3,23,34]. Prophylactic cover for labor and delivery is not normally required in carriers of hemophilia A as their FVIII levels is usually normalized at term. However, the individual's hemostatic response to pregnancy can be variable and a significant proportion, particularly those with a severe deficiency, may still have a low factor level (<50 IU/dL) at term [6,23,34]. Since FIX levels do not increase significantly during pregnancy, carriers of hemophilia B are likely to need hemostatic support for labor and delivery. If treatment is required in carriers of either hemophilia A or B, it should start at the onset of labor with the aim of raising factor level to >50 IU/dL. This level should be maintained for at least 3 days after vaginal delivery and at least 5 days after cesarean section [35,36]. Recombinant products are regarded as the treatment of choice when factor replacement is required [43]. Desmopressin can also be used in carriers of hemophilia A, but carriers of hemophilia B do not respond to this agent. Tranexamic acid is used safely for those with borderline levels.

The use of regional analgesia/anesthesia in the presence of bleeding disorders has been controversial because of its potential risk of epidural or spinal hemorrhage/hematoma that can lead to permanent neurologic damage. However, regional block is not contraindicated in these women provided their coagulation defects have normalized, either spontaneously during pregnancy or following adequate hemostatic cover [44]. Regional block was performed in 31 pregnancies among carriers of hemophilia in two UK series without any complications [23,34]. It is recommended that regional block in carriers of hemophilia should be performed by an experienced anesthetist using a midline approach and analgesia produced with the lowest concentration of local anesthetic agents so as to preserve motor function [44,45]. In addition, regular neurologic examination should be carried out until the anesthetic has worn off to allow timely recognition of any complications. If the extent or the duration of the block is more than what one would expect, immediate assessment with magnetic resonance imaging should be performed to verify the diagnosis [44,45]. More importantly, the decision regarding the use of regional block in carriers of hemophilia must be planned antenatally following joint assessment by hematologists, anesthetists, and obstetricians. The mother should be fully counseled about the risks and benefits of regional block and its alternatives.

Postpartum hemorrhage

Postpartum hemorrhage (PPH) continues to be the major cause of maternal morbidity and mortality worldwide. It is generally classified as primary or secondary PPH. Primary PPH is traditionally defined as a blood loss of more than 500 mL (or 1000 mL for severe PPH) in the first 24 h after delivery, while secondary PPH refers to excessive bleeding occurring between 24 h and 6 weeks post delivery. The risk of PPH is increased among carries of hemophilia compared with the general population [23,34]. As FVIII or FIX activity has a significant influence on the risk of bleeding among carriers of hemophilia, most of the significant PPH occur in those with factor levels below 50 IU/dL [23,34,46]. Therefore, it is recommended that FVIII or FIX should be maintained above 50 IU/dL for at least 3 days following vaginal delivery and 5 days if cesarean section has been performed to minimize the risk of primary and secondary PPH. Uterine atony is the most common cause of PPH. Prevention of this complication after delivery by adopting an active management of third stage of labor (including prophylactic use of uterotonic such as oxytocin after delivery of the baby, early cord clamping and controlled cord traction of the umbilical cord) can reduce the risk and magnitude of PPH in the mother. Carriers of hemophilia are also at risk of bleeding from perineal trauma and hematoma [6]. Therefore, care must also be taken to minimize maternal genital and perineal trauma during vaginal delivery to prevent this complication [6,35] and meticulous hemostasis should be practiced during surgery to minimize blood loss. Since the pregnancy-induced rise in clotting factors falls after delivery, carriers of hemophilia are also at risk of prolonged

Table 41.2 General principles in the management of labor and delivery in carriers of haemophilia.

Multidisciplinary approach
Group and save, full blood count and coagulation screen at the onset of labor. Relevant clotting factor assay for those with low third trimester level who require treatment
Prophylactic treatment is required when the relevant factor level is below 50 IU/dL. The hemostatic level should be maintained for at least 3 days after vaginal delivery and at least 5 days after cesarean section
Regional analgesia/anesthesia should only be considered after careful risk assessment and if the factor level and coagulation screen are normal
Avoid invasive intrapartum fetal monitoring techniques, prolonged labor, and traumatic instrumental deliveries if the fetus is at risk
Active management of third stage
Obtain cord blood sample for assessment of neonatal coagulation status in neonates at risk of moderate to severe inherited bleeding disorder
Avoid intramuscular injection in neonates at risk until the coagulation status is known—give oral vitamin K and immunization through intradermal or subcutaneous route

or intermittent secondary PPH. They should be advised of this potential complication, and in cases of heavy lochia, tranexamic acid can be used to reduce blood loss. Combined oral contraceptive pills, if not contraindicated, are also an option for preventing excessive bleeding in the late postpartum period. Guideline for management of carriers of hemophilia is presented in Table 41.2. Adopting these guidelines has been shown to minimize the risk of bleeding in the mother and neonate [23].

Rare bleeding disorders

Other inherited deficiencies of coagulation factors that may result in bleeding include afibrinogenemia, hypoprothrombinemia, and deficiencies of factor V, combined FV, and FVIII, FVII, FX, FXI, and FXIII. These disorders are inherited as autosomal recessive traits and are generally rarer than hemophilia A and B. However, in parts of the world where consanguineous marriage is frequent, recessively inherited coagulation disorders are more common. Clinically, they are expressed in homozygotes or compound heterozygotes.

Menorrhagia is a common symptom in women with rare inherited coagulation disorders and should be managed in a similar way as in carriers of hemophilia A and B.

In general, a mutation in the DNA can be identified in the genes encoding the relevant coagulation factor. For each coagulation defect there are multiple mutations, but these are unique for any given family. Prenatal diagnosis is possible for couples who have presented with an affected child. Primary prevention could be achieved by discouraging consanguineous marriage but the cultural, religious, and economic roots of this practice are deep in many cultures.

Fibrinogen and FXIII play an essential role in placental implantation and the continuation of pregnancy [47]. Deficiency of fibrinogen and FXIII are both found to be strongly associated with increased risk of recurrent miscarriages and placental abruption resulting in fetal loss or premature delivery. Replacement therapy is therefore required to be commenced as early as possible and continued throughout the pregnancy in women with these factor deficiencies [48].

The general principles of management of pregnancy and delivery in carriers of rare bleeding disorders are similar to those in carriers of hemophilia A and B. However, the available therapies are limited and are plasma derived with the exception of recombinant VIIa which may be used to treat FVII deficiency [48].

Hemostatic agents and pregnancy

The hemostatic agents used for women with inherited bleeding disorders during pregnancy are in principle similar to those used outside pregnancy. Currently available plasma-derived clotting factor concentrates are treated with virucidal methods which eliminate the risk of transmitting HIV and the hepatitis B and C viruses. However, they have the potential to transmit hepatitis A, parvovirus B19, and any unknown infection [49,50]. Although parvovirus is not normally a serious infection in nonimmunocompromised adults, fetal infection may result in hydrops fetalis and fetal death. Therefore, nonplasma-derived products, if available, are generally regarded as the treatment of choice. They include desmopressin, tranexamic acid, and recombinant products, which carry no or negligible risk of infection. Cryoprecipitate is not virally inactivated, hence should not be used during pregnancy owing to the small risk of transmission of viral or other blood-borne infections, unless other treatment modalities are not available or have failed.

References

1 Rizza CR, Rhymes IL, Austen DE, Kernoff PB, Aroni SA. Detection of carriers of haemophilia: a "blind" study. *Br J Haematol* 1975; **30**: 447–56.

2 Lyon MF. Chromatin and gene action in the mammalian X-chromosome. *Am J Hum Genet* 1962; **14**: 135–48.

3 Plug I, Mauser-Bunschoten EP, Bröcker-Vriends AH, *et al*. Bleeding in carriers of haemophilia. *Blood* 2008; **111**: 1811–15.

4 Lee CA, Chi C, Shiltagh N, *et al*. Review of a multidisciplinary clinic for women with inherited bleeding disorders. *Haemophilia* 2009; **15**: 359–60.

5 Kadir RA, Economides DL, Sabin CA, Pollard D, Lee CA. Assessment of menstrual blood loss and gynaecological problems in patients with inherited bleeding disorders. *Haemophilia* 1999; **5**: 40–8.

6 Greer IA, Lowe GD, Walker JJ, Forbes CD. Haemorrhagic problems in obstetrics and gynaecology in patients with congenital coagulopathies. *Br J Obstet Gynaecol* 1991; **98**: 909–18.

7 Kadir RA, Sabin CA, Pollard D, Lee CA, Economides DL. Quality of life during menstruation in patients with inherited bleeding disorders. *Haemophilia* 1998; **4**: 836–41.

8 Kouides PA, Phatak PD, Burkart P, *et al*. Gynaecological and obstetrical morbidity in women with type I von Willebrand disease: results of a patient survey. *Haemophilia* 2000; **6**: 643–8.

9 Kingman CE, Kadir RA, Lee CA, Economides DL. The use of levonorgestrel-releasing intrauterine system for treatment of menorrhagia in women with inherited bleeding disorders. *BJOG* 2004; **111**: 1425–8.

10 Chi C, Shiltagh N, Chase A, Griffioen A, Kadir RA. Levonorgestrel-releasing intrauterine system for the management of menorrhagia in women with inherited bleeding disorders: long term follow-up. *Thromb Res* 2007; **119**(Suppl. 1): S101.

11 Miller R. Genetic Counselling for Haemophilia. WFH treatment monograph 2002; No. 25.

12 Ludlam CA, Pasi KJ, Bolton-Maggs P, *et al*. A framework for genetic service provision for haemophilia and other inherited bleeding disorders. *Haemophilia* 2005; **11**: 145–63.

13 Ljund R, Petrini P, Nilsson IM. Diagnostic symptoms of severe and moderate haemophilia A and B—a survey of 140 cases. *Acta Paediatr Scand* 1990; **79**: 196–200.

14 Dunn NF, Miller R, Griffioen A, Lee CA. Carrier testing in haemophilia A and B: adult carriers and their partners' experiences and their views on the testing of young females. *Haemophilia* 2008; **14**: 584–92.

15 The United Nations Convention on the Rights of the Child 20. xi.1989:TS 44:Cm 1976.

16 World Health Organization. *Proposed International Guidelines on Ethical Issues in Medical Genetics and Genetic Services*. Geneva: World Health Organization, 1998.

17 BMA. *Consent, Rights and Choices in Health Care for Children and Young People*. London: BMJ Books, 2001.

18 Borry P, Fryns J-P, Schotsmans P, Dierickx K. Carrier testing in minors: a systematic review of guidelines and position papers. *Eur J Hum Genet* 2006; **14**: 133–8.

19 Antonarakis SE, Rossiter JP, Young M, *et al*. Factor VIII gene inversions in severe hemophilia A: results of an international consortium study. *Blood* 1995; **86**: 2206–12.

20 Giannelli F, Green PM, Somner S. Haemophilia B (sixth edition); a database of point mutations and short editions and deletions. *Nucleic Acids Res* 1996; **24**: 103–18.

21 Kadir RA, Sabin CA, Goldman E, Pollard D, Economides DL, Lee CA. Reproductive choices of women in families with haemophilia. *Haemophilia* 2000; **6**: 33–40.

22 Varekamp I, Suurmeijer TP, Brocker-Vriends AH, *et al*. Carrier testing and prenatal diagnosis for hemophilia; experiences and attitudes of 549 potential and obligate carriers. *Am J Med Genet* 1990; **37**: 147–54.

23 Chi C, Lee CA, Shiltagh N, Khan A, Pollard D, Kadir RA. Pregnancy in carriers of haemophilia. *Haemophilia* 2008; **14**: 56–64.

24 Tedgard U, Ljung R, McNeil TF. Reproductive choices of haemophilia carriers. *Br J Haematol* 1999; **106**: 421–6.

25 Chi C, Hyett JA, Finning KM, Lee CA, Kadir RA. Non-invasive first trimester determination of fetal gender: a new approach for prenatal diagnosis of haemophilia. *BJOG* 2006; **113**: 239–42.

26 Mujezinovic F, Alfirevic Z. Procedure-related complications of amniocentesis and chorionic villous sampling: a systematic review. *Obstet Gynecol* 2007; **110**: 687–94.

27 Firth HV, Boyd PA, Chamberlain PF, *et al*. Analysis of limb reduction defects in babies exposed to chorionic villus sampling. *Lancet* 1994; **343**: 1069–71.

28 RCOG. Amniocentesis and chorionic villus sampling. Guideline No. 8., 2005.

29 Tabor A, Philip J, Madsen M, *et al*. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* 1986; **1**: 1287–93.

30 Buscaglia M, Ghisoni L, Bellotti M, *et al*. Percutaneous umbilical blood sampling: indication changes and procedure loss rate in a nine years' experience. *Fetal Diagn Ther* 1996; **11**: 106–13.

31 Whitlow BJ, Lazanakis MS, Economides DL. The sonographic identification of fetal gender from 11–14 weeks of gestation. *Ultrasound Obstet Gynecol* 1999; **13**: 301–4.

32 Costa JM, Benachi A, Gautier E, *et al*. First trimester fetal sex determination in maternal serum using real-time PCR. *Prenatal Diagn* 2001; **21**: 1070–4.

33 Honda H, Miharu N, Ohasi Y, *et al*. Fetal gender determination in early pregnancy through qualitative and quantitative analysis of fetal DNA in maternal serum. *Hum Genet* 2002; **110**: 75–79.

34 Kadir RA, Economides DL, Braithwaite J, Goldman E, Lee CA. The obstetric experience of carriers of haemophilia. *Br J Obstet Gynaecol* 1997; **104**: 803–10.

35 Lee CA, Chi C, Pavord SR, *et al*.; UK Haemophilia Centre Doctors' Organization. The obstetric and gynaecological management of women with inherited bleeding disorders—review with guidelines produced by a taskforce of UK Haemophilia Centre Doctors' Organization. *Haemophilia* 2006; **12**: 301–36.

36 Walker ID, Walker JJ, Colvin BT, *et al*. Investigation and management of haemorrhagic disorders in pregnancy. Haemostasis and Thrombosis Task Force. *J Clin Pathol* 1994; **47**: 100–8.

37 Kulkarni R, Lusher JM, Henry RC, Kallen DJ. Current practices regarding newborn intracranial haemorrhage and obstetrical care and mode of delivery of pregnant haemophilia carriers: a survey of obstetricians, neonatologists and haematologists in the United States, on behalf of the National Hemophilia Foundation's Medical and Scientific Advisory Council. *Haemophilia* 1999; **5**: 410–15.

38 Kulkarni R, Lusher J. Perinatal management of newborns with haemophilia. *Br J Haematol* 2001; **112**: 264–74.

39 Klinge J, Auberger K, Auerswald G, Brackmann HH, Mauz-Korholz Ch, Kreuz W, and The paediatric group of the German Society of Thrombosis and Haemostasis. Prevalence and outcome of intracranial haemorrhage in haemophiliacs—a survey of the paediatric group of the German Society of Thrombosis and Haemostasis (GTH). *Eur J Pediatr* 1999; **158**(Suppl. 3): S162–5.

40 Kulkarni R, Lusher JM. Intracranial and extracranial hemorrhages in newborns with haemophilia: a review of the literatures. *J Pediatr Hematol Oncol* 1999; **21**: 289–95.

41 Ljung R, Lindgren AC, Petrini P, Tengborn L. Normal vaginal delivery is to be recommended for haemophilia carrier gravidae. *Acta Paediatr* 1994; **83**: 609–11.

42 Kulkarni A, Riddell A, Lee CA, Kadir RA. Assessment of factor VIII and von Willebrand factor levels in cord blood. RCOG 6th International Scientific Meeting, 27–30 September 2005, Cairo, Egypt.

43 United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO). Guidelines on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders. *Haemophilia* 2003; **9**: 1–23.

44 Chi C, Lee CA, England A, Hingorani J, Paintsil J, Kadir RA. Obstetric analgesia and anaesthesia in Women with inherited bleeding disorders. *Thromb Haemost* 2009; **101**: 1104–11.

45 Dhar P, Abramovitz S, DiMichele D, Gibb CB, Gadalla F. Management of pregnancy in a patient with severe haemophilia A. *Br J Anaesth* 2003; **91**: 432–5.
46 Mauser Bunschoten EP, van Houwelingen JC, Sjamsoedin Visser EJ, *et al*. Bleeding symptoms in carriers of hemophilia A and B. *Thromb Haemost* 1988; **59**: 349–52.
47 Inbal A, Muszbek L. Coagulation factor deficiencies and pregnancy loss. *Semin Thromb Hemost* 2003; **29**: 171–4.
48 Kadir RA, Chi C, Bolton-Maggs P. Pregnancy and rare bleeding disorders. *Haemophilia* 2009; **15**: 990–1005.
49 Azzi A, Ciappi S, Zakvrzewska K, *et al*. Human parvovirus B19 infection in hemophiliacs first infused with two high-purity, virally attenuated factor VIII concentrates. *Am J Hematol* 1992; **39**: 228–30.
50 Mannucci PM, Gdovin S, Gringeri A, *et al*. Transmission of hepatitis A to patients with hemophilia by factor VIII concentrates treated with organic solvent and detergent to inactivate viruses. The Italian Collaborative Group. *Ann Intern Med* 1994; **120**: 1–7.

42 von Willebrand disease: molecular aspects

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Introduction

von Willebrand disease (VWD) is a common inherited mucocutaneous bleeding disorder. It results from deficient or defective plasma von Willebrand factor (VWF) and can be divided into three disease types. Types 1 and 3 are a partial and virtually complete quantitative deficiency of VWF respectively, whereas type 2 comprises qualitative defects [1]. VWF is a large, multidomain protein with repeated domains in the order S–D1–D2–D′–D3–A1–A2–A3–D4–C1–C2–CK. Dimerization followed by multimerization of VWF during biosynthesis is essential for normal function. VWF is produced in endothelial cells and megakaryocytes and is present in the plasma as multimers formed from 10 to >40 VWF monomers [1]. It is cleaved by the metalloprotease ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin motifs) which reduces the size of the largest high-molecular-weight (HMW) multimers.

von Willebrand factor plays two important roles in hemostasis. It binds to collagen in exposed subendothelium, resulting in a conformational change that enables it to subsequently aggregate platelets at sites of blood vessel damage, through binding to platelet glycoproteins Gp1bα and GpIIbIIIa. Additionally, it carries and protects coagulation factor VIII, protecting it from premature proteolysis.

The VWF gene (*VWF*), located at the tip of chromosome 12 (12p13.3), comprises 52 exons, of which exons 2–52 encode VWF protein [2]. *VWF* has a partial pseudogene (*VWFP*) located on chromosome 22 [3] (22q11.22–11.23). *VWFP* is 97% similar in sequence to exons 23–34 of *VWF*, complicating genetic analysis of this region of the functional gene and contributing to gene conversion mutations.

One in 10 000 individuals in the population have clinically significant bleeding resulting from VWD [4], but the disorder may affect between 1 in 100 and 1 in 1000 individuals [5,6]. Up to 75% of cases have type 1 VWD, 20–25% have type 2, and up to 5% have type 3 disease. Recessively inherited type 3 VWD is more common where consanguineous partnerships are prevalent, affecting 0.5–5/10^6 population [7].

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The molecular genetics of type 2 and 3 VWD have been investigated for 20 years and the type and location of mutations responsible are relatively well established [8–10]. Type 1 VWD had been studied much less extensively until three recent multicenter studies investigated large patient cohorts [11–13].

This chapter summarizes knowledge of the molecular basis of VWD and then examines features of the gene and protein that result in mutation and relate these to the different VWD disease types. Sequence variation detailed on the International Society on Thrombosis and Haemostasis Scientific and Standardisation Committee on VWF (ISTH-SSC on VWF) mutation and polymorphism database (VWFdb) [14] was used to summarize current information on VWF mutation.

Mutations responsible for von Willebrand disease

Type 3 von Willebrand disease

Mutations affecting both *VWF* alleles are required for this rare recessively inherited VWD type to occur, where patients have virtually no detectable VWF. The majority of mutations (83% on the VWFdb) result in a null allele (resulting in little or no VWF expression), whereas a smaller proportion result in missense mutations (17%; Figure 42.1). A variety of different mutation types cause null alleles, including nonsense mutations introducing a premature stop codon; large deletions of an exon or more; small deletions, insertions or duplications, commonly affecting only one or two nucleotides; and splice site mutations, disrupting normal processing of the *VWF* mRNA. Missense mutations affect dimerization and multimerization or result in intracellular retention and are mostly located in exons 3–11 and 37–52 (Figure 42.2). Heterozygous relatives of affected individuals are often asymptomatic; the product of the mutant allele does not generally interfere with VWF produced from the normal allele.

Type 1 von Willebrand disease

A partial quantitative deficiency of VWF with a normal ratio of functional activity (VWF:RCo) to quantity of VWF protein

(VWF:Ag) and essentially normal HMW multimers characterize type 1 VWD. A heterogeneous array of mutation types and disease mechanisms that are only beginning to be understood are responsible. Mutations have only been identified in about 60% of patients examined by three large multicenter studies undertaken in Canada, Europe, and the UK [11–13]. Index cases (ICs) with lowest VWF levels plus minor multimer abnormalities in some instances had a detectable *VWF* mutation in virtually all cases, whereas of those with VWF levels close to the bottom of the normal range (50–200% of the population mean) only about 50% had a *VWF* mutation. A total of 10–15% of ICs had more than one mutation and VWF levels in these individuals tended to be lower than those with a single heterozygous mutation [12]. Missense mutations predominate, but a large range of mutation types was seen, including those resulting in a null allele and candidate promoter mutations. The same mutation categories are seen as in type 3 VWD, but in very different proportions (Figure 42.1). Two common mutations exemplify type 1 VWD; the fully penetrant dominant negative p.Arg1205His identified in 6% of ICs [11–13] results in very low VWF levels, where all individuals inheriting the mutation are affected by VWD. p.Tyr1584Cys, identified in 13% of IC results in incompletely penetrant VWD, where VWD diagnosis is more common in individuals who have coinherited ABO blood group O. This, and similar mutations, could be regarded as risk factors for bleeding. Blood group O is much more frequent in type 1 VWD cases (65% of European ICs) than in the normal population (38% [12]) and contributes to the reduced plasma VWF level.

Type 2 von Willebrand disease

Type 2 VWD comprises qualitative disorders. These predominantly result from missense mutations and occasionally from in-frame deletions or insertions which result in a VWF protein of slightly altered size. Mutation location is represented in Figure 42.2.

Type 2A

Mutations in this VWD type lead to decreased VWF-dependent platelet adhesion and a deficiency of HMW multimers. Dominantly inherited missense alterations in or close to the A2 domain enhance VWF cleavage by the metalloprotease ADAMTS13. Mutations can be classified into two groups: group I mutations enhance VWF proteolysis whilst impairing intracellular multimer assembly, whereas group II mutations enhance proteolysis without affecting multimer secretion. These mutations comprise at least three-fourths of type 2A VWD; smaller proportions of mutations are located in the D2, D3, and CK domains. Mutations affecting CK domain disulfide bonding required for VWF dimerization yield aberrant multimer species terminated by VWF monomers. 2A(IID) VWD results from these mutations centered around Cys2771–Cys2773. Mutations in the D2 domain can interfere with intersubunit disulfide bonding; reported mutations in exons 11–15 (residues 404–625) result in 2A(IIC) VWD. D3 mutations impede disulfide bond formation between subunits and result in subtype 2A(IIE), characterized by "smeary" multimer patterns on gel electrophoresis. D2 and some CK mutations are recessively inherited, requiring a second similar missense mutation or null allele for VWD to occur.

Type 2B

Mutations in this dominantly inherited VWD type have increased affinity for platelet GpIbα. Following secretion, VWF binds spontaneously to platelets which facilitates cleavage by ADAMTS13 and can lead to loss of HMW multimers. Mutations are all within or close to the A1 domain and result in its conformational change. All are dominantly inherited missense changes with the exception of one reported in-frame insertion. The VWFdb [14] lists 23 missense alterations affecting only 13 different amino acids between codons 1266 and 1461.

Type 2M

Mutations in 2M VWD have decreased VWF-dependent platelet adhesion without deficiency of HMW multimers. Dominantly inherited mutations that disrupt VWF binding to platelets or to subendothelium are responsible. This subtype is difficult to diagnose. Good measurements of ability of VWF to bind GpIbα (VWF:RCo) and collagen (VWF:CB) [relative

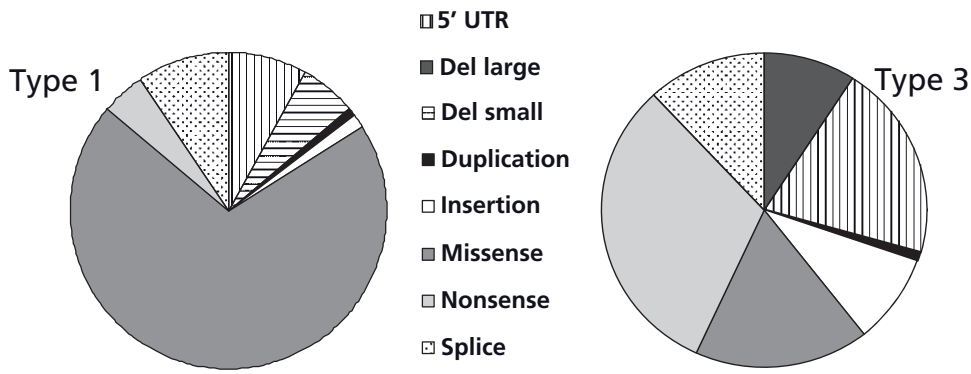

Figure 42.1 Proportion of different mutation types reported in type 1 and type 3 von Willebrand disease. Pie charts represent mutation types reported on the VWFdb in December 2008, divided into eight different mutation categories. Large deletions are of at least one exon. Del, deletion.

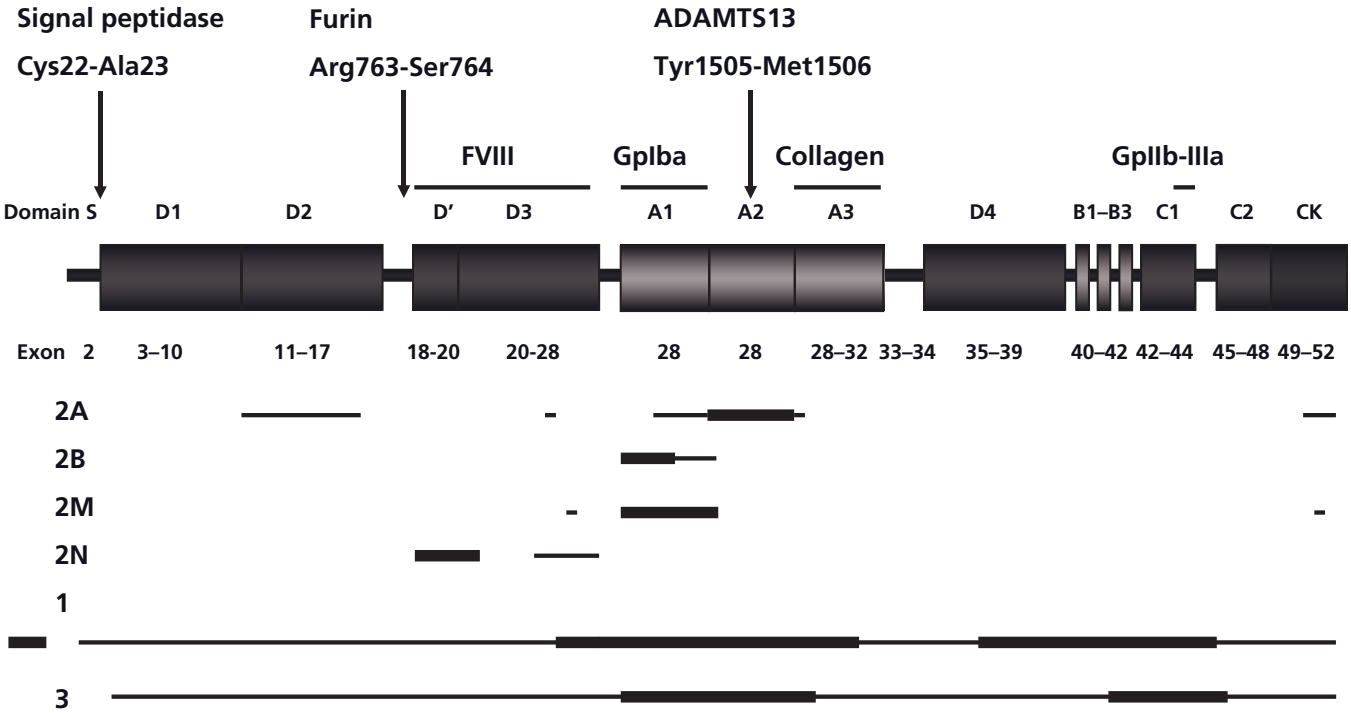

Figure 42.2 VWF gene and protein showing ligand-binding sites, cleavage sites for signal peptidase, Furin and ADAMTS13 plus sites of mutation reported to the VWFdb. Thin lines represent the extent of mutations reported; thick lines represent most common mutation sites. 5′ UTR sequence variants reported in type 1 VWD are indicated by the thick line extending to the left of the VWF protein coding region.

to the quantity of VWF protein (VWF:Ag)] and a high-resolution multimer profile to demonstrate presence of HMW multimer are required. Mutations reported to date are predominantly in the A1 domain (codons 1266–1467), with isolated reports in the D3, A2, and CK domains. Mutation in the A3 domain affecting VWF-collagen binding (p.Ser1731Thr) has only recently been recognized [15].

Type 2N

Mutations in VWD type 2N markedly decrease binding affinity for FVIII and result in low plasma FVIII, whilst VWF levels can be within the normal range. This disease type can be challenging to discriminate from mild hemophilia A in males and hemophilia A carriership in females. The FVIII binding domain was mapped to the first 272 residues of mature VWF (764–1035) [1], but mutations indicate that the area of VWF that can interfere with FVIII binding extends slightly further (760–1225) [14].

Two mutations are required for this recessively inherited VWD subtype: it can result from two missense mutations, both of which disrupt FVIII binding, or more commonly a missense plus a null allele. Mutations are predominantly in exons 18–20 (79% of mutations on the VWFdb) with the remainder being in exons 17 (p.Arg760Cys), 24, 25, and 27 (p.Cys1225Gly). A small proportion of mutations, notably those leading to loss of a cysteine residue, also result in a loss of HMW multimers (p.Cys788Arg, p.Asp879Asn, p.Cys1060Arg, p.Cys1225Gly) [16,17], whereas multimers in most cases are normal.

Mechanisms of mutation

Table 42.1 summarizes some of the characteristics of the VWF gene and protein which result in susceptibility to particular mutations. Many of these are common to other genes and proteins, whereas a few (such as gene conversion) represent more unusual mutation types. Some are described below.

Gene size and GC content

VWF is a relatively large gene and thus presents a large target for mutation. The cDNA comprises 58% G + C residues. This slightly higher than average GC content increases the proportion of CpG dinucleotides present. These are a well-established hotspot for point mutations resulting particularly from C>T and G>A mutations [18]. *VWF* exon 28 exemplifies the excess rate at which mutations affect CpG sequences; CpG constitute only 8% of exon 28 sequence (58 CpG in 1378 bp) but account for 43% of its mutations. Arginine, predominantly encoded by CGN codons (N = any nucleotide), is thus the most mutated VWF amino acid, being affected by 26% of all mutations on

Table 42.1 von Willebrand factor (VWF) features resulting in susceptibility to particular mutations.

Feature	Detail	Result	Mutation location
Large gene size	Coding and flanking regions >180 kb	*VWF* presents a large target for mutations	*VWF*
Repetitive elements within introns	>14 Alu repeats within *VWF*	Homologous recombination can result in large gene deletions	5′ UTR and introns
Small nucleotide runs	Direct (particularly polypyrimidine) and inverted repeats susceptible to DNA polymerase replication errors	Nucleotide repeat sequences common site of small deletion and insertion mutations	Repeated nucleotides
CpG dinucleotides	Deamination of 5-methylcytosine to thymine results in C>T or G>A mutations being common	Many point mutations occur at CpG dinucleotides. Mutations affecting arginine codons (CGN) over-represented	CpG sequences
Partial pseudogene	*VWFP* 97% similar to exons 23–34 and located on separate chromosome (22). *VWF* close to chromosome 12 telomere. Chi-like sequences in *VWF* and *VWFP*	Gene conversions affecting intron 27 and exon 28 are common	3′ end of intron 27 to 5′ end of exon 28
Transcription factor binding sites (TFBS)	Disruption of TFBS	Lack of/reduced mRNA transcription from one allele	5′ UTR
Splice sites	Disruption of normal splicing	Can result in intron inclusion, often leading to a null allele or exon skipping, sometimes resulting in an in-frame deletion	Sequence surrounding splice junctions
Signal peptide cleavage by signal peptidase	Disruption/loss of cleavage	May result in VWF misfolding because of signal peptide retention	Not yet reported
VWF dimerization	VWF forms tail to tail dimers through disulfide bonds in the CK domain	Point mutation, particularly missense changes can disrupt VWF dimerization [2A (IID)]	CK domain
VWF multimerization	VWF forms head-to-head multimers through disulfide bonds between D3 domains	Point mutation, particularly missense changes can disrupt VWF multimerization [2A (IIE and IIC)]	D3 and D2 domains
Propeptide cleavage by Furin	Lack of propeptide cleavage from mature VWF	VWF–FVIII binding disrupted by steric hindrance	D′ domain
Disulfide bonds	VWF contains 163 cysteines, all involved in intra- or interchain disulfide bonds	Cys loss or gain disrupts normal disulfide pairing. Many of these mutations result in multimer abnormalities or lack of multimerization	Cys residues, other residues which can be mutated to Cys
VWF cleavage by ADAMTS13	Enhancement of susceptibility to cleavage by VWF-cleaving metalloprotease, ADAMTS13	Missense changes in A domains enhance accessibility of p.Tyr1505–Met 1506 bond to cleavage	A2, also A1 and A3
VWF ligand-binding sites	Disruption/enhancement of VWF binding to ligands including GpIbα, FVIII and collagen	Point mutations, particularly missense changes and in-frame deletions/ insertions disrupt/enhance VWF function	Functional sites in A, D′ and D3 domains
VWF clearance from plasma	Enhanced VWF clearance from plasma	Missense changes, particularly in the D3 domain, enhance VWF clearance by cells including macrophages [37]	Predominantly D3; D4 also reported

VWFdb. Mutation affecting CpG dinucleotides result in all VWD types.

Large deletions and Alu repeats

Large deletions (deletions of at least one *VWF* exon) were the first VWD mutation types to be identified. Deletions were initially characterized using Southern blotting; hence, mutation extent is imprecisely known and mutation mechanism was not ascertained in these cases. More recently identified mutations have been precisely mapped. For example, homologous unequal recombination between Alu repeats [19] (small mobile genetic elements very frequent in the human genome, initially recognized by the restriction enzyme Alu I) have been

shown to be involved in the pathogenesis of some phenotypes [20–22]. At least 14 Alu repeats are present within *VWF* introns and promoter [2]. Large deletions are probably underrecognized owing to the difficulty of identifying them in the presence of a nondeleted allele. Most result in nonexpression of VWF. They constitute 9% of mutations in type 3 VWD on the VWFdb and also contribute to type 1 disease [22].

Repeated nucleotides

DNA polymerase makes errors more frequently where a motif is repeated in the DNA sequence. Error rate is also influenced by the local DNA sequence environment; direct or inverted repeats often flank the mutated nucleotides [19]. *VWF* has several locations where the nucleotide sequence is repeated and these may be characterized by deletion or insertion mutations resulting from slipped strand mispairing [19]. The best-known example is a run of six cytosine residues in exon 18 (c.2430–35) where one C residue is deleted [23]. Deletions or insertions of a C nucleotide occur more frequently than those of G, T, or A nucleotides in *VWF* and are often seen in polypyrimidine sequences (C and T residues), e.g., deletion of C from CTCCT (c.3072delC) [19]. Other examples occur in direct repeats, e.g., deletion of AAG from the sequence AAGAAGAAGAAG $(AAG)_4$ (c.4222–24delAAG) and deletion of GT from $(GT)_4$ (c.7296–97delGT). Small deletions are twice as frequent as small insertions. One-fourth of these mutations result in in-frame alterations to the VWF protein (contributing to type 1 and 2 VWD), whereas the remainder result in a null allele.

Table 42.2 VWF variants reported to result from gene conversion.

Nucleotide substitution	Amino acid substitution	Patients with change in VWF literature, n = 36 cases[a]
c.3675–45C>T	NA	1
c.3675–36C>T	NA	1
c.3686T>G	p.Val1229Gly	8
c.3692A>C	p.Asn1231Thr	9
c.3735G>A	p.Val1245	4
c.3789G>A	p.Ser1263	12
c.3797C>T (C>A)	p.Pro1266Leu (Pro>Gln)	19
c.3835G>A	p.Val1279Ile	16
c.3931C>T	p.Gln1311X	11
c.3951C>T	p.Ala1317	12
c.4027A>G	p.Ile1343Val	6
c.4079T>C	p.Val1360Ala	6
c.4105T>A	p.Phe1369Ile	6

[a]From refs 11, 12, 14, 25–29, and 38–41.

von Willebrand factor pseudogene and gene conversion

Gene conversion is being recognized as a reasonably common mutation mechanism in VWD (Table 42.2). A short stretch of the *VWFP* sequence invades the gene sequence and replaces it [24]. Several factors appear to predispose to conversion: Chi-like 8-bp sequences in intron 27 and exon 28; proximity of *VWF* to the chromosome 12 telomere; and *VWFP* residing on a separate chromosome [24]. Review of the VWF literature (December 2008) identified 36 patients in 13 publications where gene conversion was deduced to be responsible for *VWF* mutation. Two consecutive *VWF* nucleotide substitutions corresponding to *VWFP* sequence are sufficient to indicate that a conversion is likely to have occurred. The longest reported conversion tracts have six consecutive nucleotide alterations, from c.3835G>A to c.4105T>A (Table 42.2). The most commonly identified mutation combinations are illustrated in Figure 42.3.

Minimum and maximum extents of conversion can be deduced from examination of *VWF* and *VWFP* sequence differences and range from 8 to 335 bp. It is possible that conversions extend further into intron 27; however, these are not detected because PCR and DNA sequencing primers do not generally extend more than about 50 bp into the intron.

von Willebrand disease phenotypes resulting from conversions depend on the range of nucleotide alterations and consequent amino acid substitutions. Those incorporating p.Val1229Gly result in reduced VWF levels and have been classified as either type 1 or 2M VWD [11,25,26]. Conversions including p.Pro1266Leu (or, less frequently, p.Pro1266Gln) result in type 2B (New York/Malmö) [27] whereas those incorporating p.Gln1311X result in a null allele and have been reported in several type 3 VWD patients [26,28,29].

5′-Untranslated region (5′-UTR) and transcription factor binding sites (TFBS)

Recent type 1 VWD studies [11–13] were the first to examine the *VWF* 5′-UTR in a significant number of patients, and several single nucleotide variants have been identified (8% type 1 cases on VWFdb). However, it is not yet known whether these affect the level of *VWF* transcription significantly. The Canadian study identified one IC with a deletion of 13 nucleotides from c. –1522 to –1510, only 48 bp 5′ of the transcription start site [11]. This mutation appears likely to affect transcription from one *VWF* allele, indicating that the 5′-UTR may be an additional site that should be examined for candidate mutations.

Cysteine residues

VWF has a high proportion of cysteine residues (8% of amino acids). All are involved in intra- or interchain disulfide bonds.

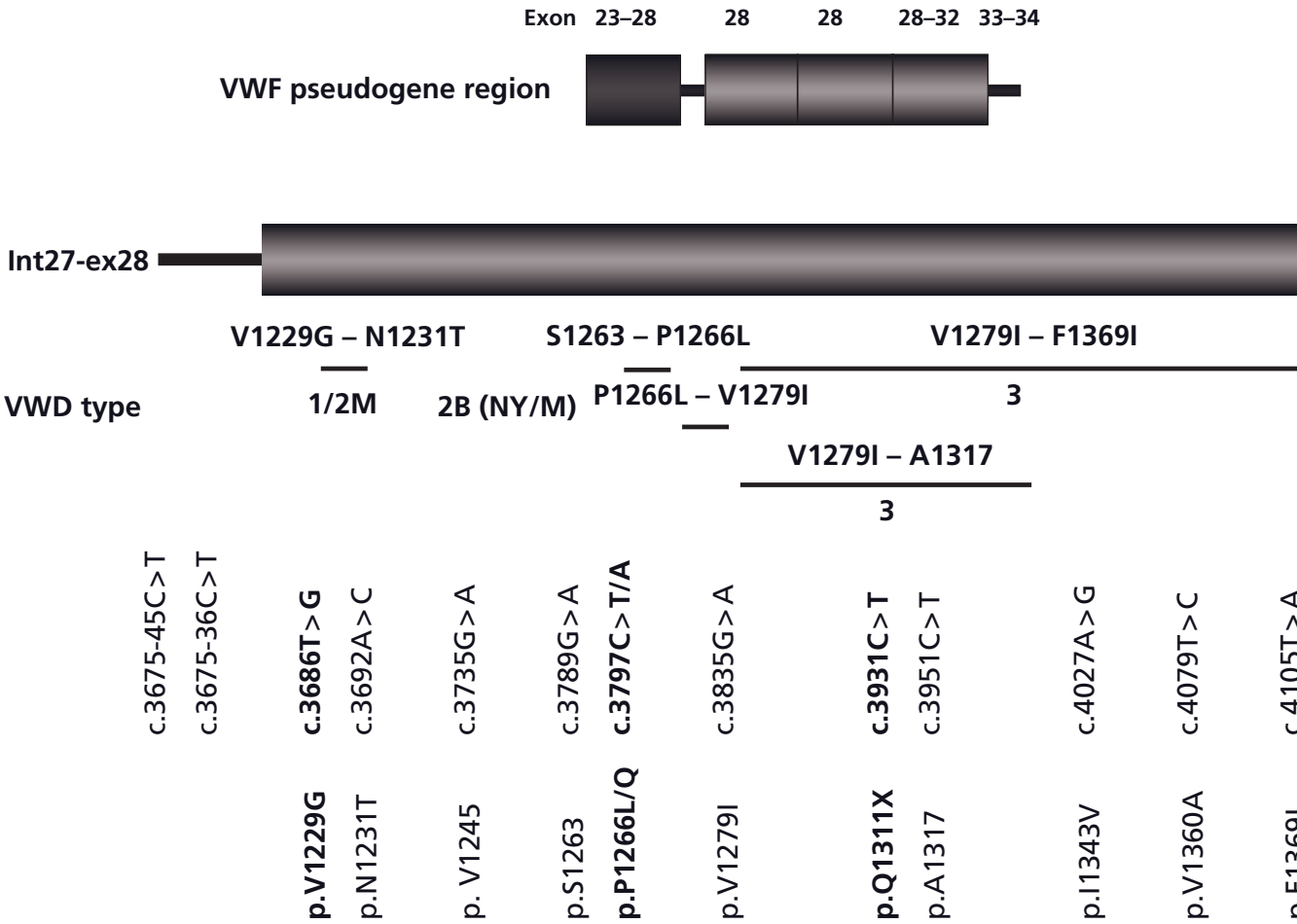

Figure 42.3 Gene conversion mutations reported in *VWF*. The top line represents the extent of the VWF coding region included in the pseudogene *VWFP*, with functional regions represented as in Figure 42.2. The second line represents the 485-bp region of intron 27–exon 28 reported to date to participate in gene conversions. Five black lines underneath represent the extent of the most commonly reported conversion events, with amino acid residues highlighting 5′ and 3′ minimal conversion length. The bottom panel represents all nucleotide and amino acid substitutions reported. Sequence alterations in bold appear to be those that dictate VWD phenotype (illustrated underneath common conversion events). *VWFP* has a single nucleotide polymorphism, both T and A alleles of which have been introduced into the gene in different patients at c.3797, resulting in either lysine or glutamine substitutions for the reference sequence proline [27].

Many, but not all, missense mutations that result in loss or gain of a cysteine residue result in multimer abnormalities. These can range from a "smeary" multimer pattern to defective dimerization or multimerization [1]. Cysteine mutations contribute to all VWD types.

von Willebrand factor clearance

von Willebrand factor has a half-life in plasma of 12–20 h [1]. This can be shortened by mutation and is also influenced by both N-linked ABO [30,31] and O-linked glycosylation [32]. Relative clearance rate can be determined by measurements of plasma VWF propeptide in comparison with mature VWF (VWFpp/VWF:Ag ratio) [33] or by monitoring VWF levels following desmopressin infusion [34]. Missense mutations, particularly those in the D3 domain, may lead to dramatically increased clearance rate. Variants affecting p.Arg1205 and p.Cys1130 identified in type 1 VWD exemplify these mutations [35,36].

Conclusion

The large multifunctional VWF protein is encoded by the extensive *VWF* gene. Both provide scope for many different mutation types, resulting in the diverse array of VWD types and mutation mechanisms responsible for them. Despite extensive molecular analyses, these remain incompletely understood.

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References

1 Sadler JE, Budde U, Eikenboom JC, *et al*. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. *J Thromb Haemost* 2006; **4**: 2103–14.

2 Mancuso DJ, Tuley EA, Westfield LA, *et al*. Structure of the gene for human von Willebrand factor. *J Biol Chem* 1989; **264**: 19514–27.

3 Mancuso DJ, Tuley EA, Westfield LA, *et al*. Human von Willebrand factor gene and pseudogene: structural analysis and differentiation by polymerase chain reaction. *Biochemistry* 1991; **30**: 253–69.

4 Bloom AL. von Willebrand factor: clinical features of inherited and acquired disorders. *Mayo Clin Proc* 1991; **66**: 743–51.

5 Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. *Blood* 1987; **69**: 454–9.

6 Werner EJ, Broxson EH, Tucker EL, *et al*. Prevalence of von Willebrand disease in children: a multiethnic study. *J Pediatr* 1993; **123**: 893–8.

7 Rodeghiero F. von Willebrand disease: still an intriguing disorder in the era of molecular medicine. *Haemophilia* 2002; **8**: 292–300.

8 Meyer D, Fressinaud E, Gaucher C, *et al*. Gene defects in 150 unrelated French cases with type 2 von Willebrand disease: from the patient to the gene. INSERM Network on Molecular Abnormalities in von Willebrand Disease. *Thromb Haemost* 1997; **78**: 451–6.

9 Eikenboom JC. Congenital von Willebrand disease type 3: clinical manifestations, pathophysiology and molecular biology. *Best Pract Res Clin Haematol* 2001; **14**: 365–79.

10 Fressinaud E, Mazurier C, Meyer D. Molecular genetics of type 2 von Willebrand disease. *Int J Hematol* 2002; **75**: 9–18.

11 James PD, Notley C, Hegadorn C, *et al*. The mutational spectrum of type 1 von Willebrand disease: Results from a Canadian cohort study. *Blood* 2007; **109**: 145–54.

12 Goodeve A, Eikenboom J, Castaman G, *et al*. Phenotype and genotype of a cohort of families historically diagnosed with type 1 von Willebrand disease in the European study, Molecular and Clinical Markers for the Diagnosis and Management of Type 1 von Willebrand Disease (MCMDM-1VWD). *Blood* 2007; **109**: 112–21.

13 Cumming A, Grundy P, Keeney S, *et al*. An investigation of the von Willebrand factor genotype in UK patients diagnosed to have type 1 von Willebrand disease. *Thromb Haemost* 2006; **96**: 630–41.

14 International Society on Thrombosis and Haemostasis Scientific and Standardization Committee VWF Information Homepage [database on the Internet]. Available at: www.vwf.group.shef.ac.uk (accessed December 1, 2008).

15 Ribba AS, Loisel I, Lavergne JM, *et al*. Ser968Thr mutation within the A3 domain of von Willebrand factor (VWF) in two related patients leads to a defective binding of VWF to collagen. *Thromb Haemost* 2001; **86**: 848–54.

16 Allen S, Abuzenadah AM, Blagg JL, *et al*. Two novel type 2N von Willebrand disease-causing mutations that result in defective factor VIII binding, multimerization, and secretion of von Willebrand factor. *Blood* 2000; **95**: 2000–7.

17 Jorieux S, Gaucher C, Goudemand J, *et al*. A novel mutation in the D3 domain of von Willebrand factor markedly decreases its ability to bind factor VIII and affects its multimerization. *Blood* 1998; **92**: 4663–70.

18 Cooper DN, Krawczak M. The mutational spectrum of single base-pair substitutions causing human genetic disease: patterns and predictions. *Hum Genet* 1990; **85**: 55–74.

19 Cooper DN. Human gene mutation in pathology and evolution. *J Inherit Metab Dis* 2002; **25**: 157–82.

20 Xie F, Wang X, Cooper DN, et al. A novel Alu-mediated 61-kb deletion of the von Willebrand factor (VWF) gene whose breakpoints co-locate with putative matrix attachment regions. *Blood Cells Mol Dis* 2006; **36**: 385–91.

21 Mohl A, Marschalek R, Masszi T, *et al*. An Alu-mediated novel large deletion is the most frequent cause of type 3 von Willebrand disease in Hungary. *J Thromb Haemost* 2008; **6**: 1729–35.

22 Sutherland MS, Cumming AM, Bowman M, *et al*. A novel deletion mutation is recurrent in von Willebrand disease types 1 and 3. *Blood* 2009; **114**: 1091–8.

23 Zhang ZP, Falk G, Blomback M, *et al*. A single cytosine deletion in exon 18 of the von Willebrand factor gene is the most common mutation in Swedish vWD type III patients. *Hum Mol Genet* 1992; **1**: 767–8.

24 Chen JM, Cooper DN, Chuzhanova N, *et al*. Gene conversion: mechanisms, evolution and human disease. *Nat Rev Genet* 2007; **8**: 762–75.

25 Eikenboom JC, Castaman G, Vos HL, *et al*. Characterization of the genetic defects in recessive type 1 and type 3 von Willebrand disease patients of Italian origin. *Thromb Haemost* 1998; **79**: 709–17.

26 Gupta PK, Adamtziki E, Budde U, *et al*. Gene conversions are a common cause of von Willebrand disease. *Br J Haematol* 2005; **130**: 752–8.

27 Baronciani L, Federici AB, Castaman G, *et al*. Prevalence of type 2b "Malmo/New York" von Willebrand disease in Italy: the role of von Willebrand factor gene conversion. *J Thromb Haemost* 2008; **6**: 887–90.

28 Surdhar GK, Enayat MS, Lawson S, *et al*. Homozygous gene conversion in von Willebrand factor gene as a cause of type 3 von Willebrand disease and predisposition to inhibitor development. *Blood* 2001; **98**: 248–50.

29 Gupta PK, Saxena R, Adamtziki E, *et al*. Genetic defects in von Willebrand disease type 3 in Indian and Greek patients. *Blood Cells Mol Dis* 2008; **41**: 219–22.

30 Davies JA, Collins PW, Hathaway LS, *et al*. von Willebrand factor: evidence for variable clearance in vivo according to Y/C1584 phenotype and ABO blood group. *J Thromb Haemost* 2008; **6**: 97–103.

31 Gallinaro L, Cattini MG, Sztukowska M, *et al*. A shorter von Willebrand factor survival in O blood group subjects explains how ABO determinants influence plasma von Willebrand factor. *Blood* 2008; **111**: 3540–5.

32 van Schooten CJ, Denis CV, Lisman T, *et al*. Variations in glycosylation of von Willebrand factor with O-linked sialylated T antigen are associated with its plasma levels. *Blood* 2007; **109**: 2430–7.

33 Haberichter SL, Balistreri M, Christopherson P, *et al*. Assay of the von Willebrand factor (VWF) propeptide to identify patients with type 1 von Willebrand disease with decreased VWF survival. *Blood* 2006; **108**: 3344–51.

34 Castaman G, Lethagen S, Federici AB, *et al*. Response to desmopressin is influenced by the genotype and phenotype in type 1 Von Willebrand Disease (VWD): results from the European study MCMDM-1VWD. *Blood* 2008; **111**: 3531–9.
35 Millar CM, Riddell AF, Brown SA, *et al*. Survival of von Willebrand factor released following DDAVP in a type 1 von Willebrand disease cohort: influence of glycosylation, proteolysis and gene mutations. *Thromb Haemost* 2008; **99**: 916–24.
36 Haberichter SL, Castaman G, Budde U, *et al*. Identification of type 1 von Willebrand disease patients with reduced von Willebrand factor survival by assay of the VWF propeptide in the European study: molecular and clinical markers for the diagnosis and management of type 1 VWD (MCMDM-1VWD). *Blood* 2008; **111**: 4979–85.
37 van Schooten CJ, Shahbazi S, Groot E, *et al*. Macrophages contribute to the cellular uptake of von Willebrand factor and factor VIII in vivo. *Blood* 2008; **112**: 1704–12.
38 Zhang ZP, Blomback M, Nyman D, *et al*. Mutations of von Willebrand factor gene in families with von Willebrand disease in the Aland Islands. *Proc Natl Acad Sci USA* 1993; **90**: 7937–40.
39 Eikenboom JC, Vink T, Briet E, *et al*. Multiple substitutions in the von Willebrand factor gene that mimic the pseudogene sequence. *Proc Natl Acad Sci USA* 1994; **91**: 2221–4.
40 Holmberg L, Dent JA, Schneppenheim R, *et al*. von Willebrand factor mutation enhancing interaction with platelets in patients with normal multimeric structure. *J Clin Invest* 1993; **91**: 2169–77.
41 Kanaji T, Okamura T, Kuroiwa M, *et al*. A sequence analysis of von Willebrand factor mRNA, gene, and pseudogene in two patients with von Willebrand disease type 2B, and an investigation of gene conversion in its gene. *Int J Hematol* 1996; **64**: 53–9.

43 von Willebrand disease: epidemiology

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In 1926 Erik von Willebrand investigated a family with a new bleeding disorder, later to become universally known as von Willebrand disease (VWD) in recognition of his pioneering discovery [1]. At that time, he was probably unaware that he was studying what would be recognized 60 years later as the most frequent inherited hemorrhagic disorder. Indeed, VWD is high in the differential diagnosis of patients presenting with mild bleeding symptoms and a prevalence up to 1% has been estimated in a large epidemiologic investigation conducted by our group in 1987 [2]. However, a clearcut diagnosis of VWD remains often difficult or elusive, owing to the wide spectrum of clinical and laboratory manifestations and to the lack of strong penetrance and expressivity in its inheritance. Unfortunately, in this regard recent molecular characterization of the disease does not provide practical guidance for diagnosis.

For these reasons, the actual prevalence of clinically significant cases of VWD is uncertain despite the ever-increasing number of diagnostic laboratory tools. Prevalence estimates are in fact critically influenced by the clinical criteria used to select subjects and also by the laboratory criteria to confirm the diagnosis. Moreover, the assessment of the bleeding history is often more difficult in the epidemiologic than in the clinical setting. Physicians must rely on the patient's bleeding history, which is confounded by personal recollection (recall bias), unless subjects have suffered from severe hemorrhages, possibly leading to hospitalization. Until recently, no clinical methods were available to objectively quantitate mild or intermediate bleeding symptoms, apart from possibly surgical bleeding and menorrhagia [3]. The number of symptoms reported by a patient may be influenced by his/her education, family setting (e.g., some symptoms may be under-reported by subjects belonging to a bleeding family) and personality, but also by the type of data ascertainment. For example, as many as 23% of Swedish girls reported three or more hemorrhagic symptoms when a self-reported questionnaire was used [4]. On the contrary, when a physician-managed questionnaire was used to query these same young women, three or more hemorrhagic symptoms were reported in fewer than 1% of normal control subjects [5]. Hence, stringent clinical criteria and standardized questionnaires to assure interobserver reproducibility have the highest likelihood of achieving complete ascertainment [6]. Thus, it is important to understand the potential pitfalls that arise in prevalence estimation of VWD in relation to its clinical presentation. The use of different methods for prevalence estimation may indeed produce diverging results, with far lower prevalence figures obtained in hospital-based investigations in comparison with population-based investigations, since different categories of subjects are considered (Figure 43.1).

Ascertainment and validity of epidemiologic data on von Willebrand disease

von Willebrand disease is usually classified into three types on the basis of clinical and laboratory phenotypes. Type 1, accounting for the large majority of cases, is represented by partial quantitative defects; type 2 by qualitative defects; and type 3 by virtual absence of von Willebrand factor (VWF) in plasma [7]. In practice, qualitative defects are suspected by measuring a significantly reduced VWF ristocetin cofactor activity to antigen ratio and confirmed by assessing the distribution of high/intermediate/low molecular weight (mw) VWF multimers or more subtle abnormalities. This allows subclassification of type 2 into 2A (lack of high and intermediate mw multimers), 2B (lack of high mw multimers), 2M (all species of multimers present), or more rare variants [8]. However, from the point of view of clinical presentation and diagnostic approach, three distinct groups of VWD patients may be considered (Table 43.1).

In the first group (group A), patients present with a lifelong history of severe to moderate bleeding symptoms, often requiring hospitalization for transfusion, replacement therapy, surgical intervention (e.g., nose packing for epistaxis, dilatation and curettage in women for menorrhagia). Iron-deficiency anemia is also common, especially in women. Laboratory investigations show VWF activity levels below or around 10 IU/dL. Linkage with a mutant VWF gene is usually complete as well as the likelihood to detect a specific VWF mutation [9]. This group of patients includes recessive type 3

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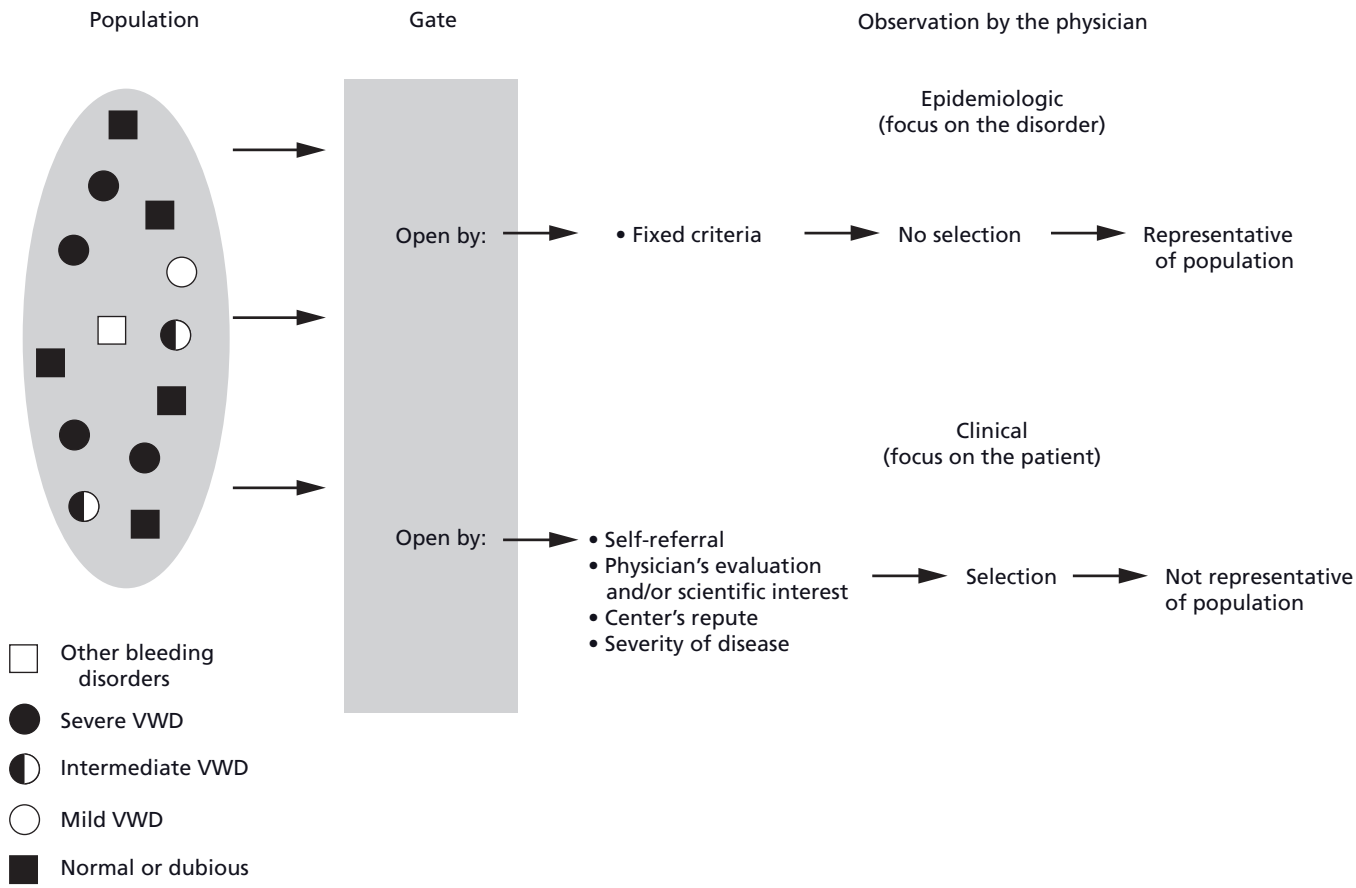

Figure 43.1 How the criteria used for the identification of patients to be investigated for von Willebrand disease affects the prevalence estimates of the disease.

Table 43.1 Classification of von Willebrand disease (VWD) according to clinical presentation and diagnostic approach.

	Severe VWD (group A)	Intermediate VWD (group B)	Mild VWD (group C)
Symptoms	Manifest bleeding	Intermediate	Mild or very mild
Cosegregation (linkage) of symptoms with low VWF/haplotype	Invariable	Variable	Inconsistent
VWF levels	About 10 IU/dL or less	About 30 IU/dL	40–50 IU/dL
Diagnosis	Easy	Repeated testing needed	Not always possible; not clinically useful in most cases
Epidemiologic ascertainment	Referral-based: appropriate	Referral-based: underestimated	Cross-sectional: overestimated

VWF, von Willebrand factor.

VWD, some dominant type 1 patients with full penetrance and expressivity (including the Vicenza type now included among type 1 VWD), and most type 2A and 2B VWD patients. Prevalence estimates for these patients may be reliably obtained from hospital-based cohorts, as it is highly unlikely that these patients have ever been referred to a specialized secondary coagulation center, at least in Western countries.

The second group of patients (group B) comprises subjects with a milder but still definite bleeding phenotype. These patients have frequent spontaneous bleeding episodes (such as mucocutaneous bleeding) and may be referred for bleeding after trauma or minor surgery, especially when mucous membranes are involved. Laboratory investigations typically show VWF activity levels around 30 IU/dL. Linkage analysis with mutations in VWF gene is consistent with an autosomal dominant disease with variable penetrance in most cases [10,11]. This group of patients comprises most subjects with type 1 and some with type 2 VWD. Prevalence estimates for these patients are partly underestimated from hospital-based cohorts, because some patients may never seek hospital advice, resulting in a falsely low prevalence. Very large, cross-sectional (population-based) investigations should be used to identify this cohort group.

The third group of patients (group C) comprises patients with a quite mild hemorrhagic phenotype (bleeding score around 1). Bleeding symptoms are occasional, sometimes absent even after trauma or minor surgery. Laboratory investigation shows VWF activity levels around 40–50 IU/dL, often requiring repeated measurements and adjustment for ABO group to achieve an accurate diagnosis. Linkage analysis fails to detect association with VWF haplotype in up to 50% of the families, indicating a possibly spurious association [12]. Family investigations and standardized diagnostic tools (e.g., bleeding questionnaire) are of utmost importance in trying to achieve a definite diagnosis. Indeed, in most group C patients, we could demonstrate by using a Bayesian approach developed by our group [13] that even in presence of two family members (including the proband) having VWF levels just below 40 IU/dL) a final odds ratio of VWD of approximately 2.0 would be produced (false-positive equals true-positive rates). This creates a need for setting more stringent clinical criteria to achieve a "useful" diagnosis, thereby avoiding a mislabeling with the stigmata of a definite genetic diagnosis subjects with a small risk of minor bleeding.

Prevalence of severe von Willebrand disease (group A VWD)

Most cases of clinically moderate to severe VWD are represented by type 3 and some cases with type 1, type 2A, and 2B VWD. The prevalence of type 3 VWD is very low, ranging from 0.1 to 5.3 per million in the population. Weiss *et al.* reported in 1982 a prevalence of 1.53 and 1.38 per million of severe VWD in Europe and North America respectively, based on the report from 195 referral centers worldwide [14]. A subsequent re-evaluation of these subjects through measurement of VWF:Ag with a highly sensitive method (immunoradiometric assay) showed a prevalence of severe VWD (defined by an antigen level below 1 IU/dL) of 0.45 per million [15]. Significant differences in the prevalence of severe VWD were present in different countries, notably with a higher prevalence in Scandinavian countries (2.4–3.12 per million) [15]. The highest prevalence of type 3 VWD was, however, observed by Berliner *et al.* [16] in Arabs, in whom consanguinity is rather frequent, with an estimated prevalence of 5.3 per million.

Prevalence of intermediate von Willebrand disease (group B VWD)

As for severe VWD, prevalence estimates for intermediate VWD are available only from hospital-based cohorts: these are calculated as the number of patients registered at a single specialized center, divided by the total population served by the center. The first data published with this methodology date back to 1984, when Nilsson estimated that there were about 530 known cases (230 families) of VWD in Sweden, corresponding to a prevalence of seven VWD patients per 100 000 inhabitants, the same figure as for hemophilia in that country [17]. This study, however, also included patients with type 3. In 1991, Bloom and Giddins [18] carried out an international survey of the prevalence of acquired immunodeficiency syndrome (AIDS) in VWD, trying to indirectly estimate the prevalence of VWD. A questionnaire was dispatched to the hemophilia center directors of 59 countries, soliciting information on the number of patients with VWD attending each center and the proportion of those treated with blood derivatives. Information was retrieved from 63% of the centers belonging to 37 countries concerning 16 664 identified patients, of whom 7534 were treated. The prevalence estimate was consequently very heterogeneous in the various countries, ranging from 3.7 to 239 cases per million inhabitants (Table 43.2). Surprisingly, the estimated prevalence for Scandinavia was about twice that reported by Nilsson [17] in the previously mentioned study. Whatever the limitations of this approach, the prevalence of patients with intermediate VWD requiring specific treatment has been estimated to range from 40 to 100 cases per million [17–20], a figure often quoted as a reliable estimation [21].

Table 43.2 Prevalence of referred VWD. Modified from [18].

Area	Population (million)	Patients reported	Corrected prevalence, per million
Scandinavia	21.5	4749	239
Rest of Europe	441	6514	23
Australasia	16	599	42
North America	237	2263	22
Israel	3.5	106	60.4
Far East	286	1673	8.1
South America	133	600	3.7
South Africa	24	80	7.4

Prevalence of mild von Willebrand disease

Four population-based studies are available (Table 43.3). Rodeghiero *et al.* [2,22] evaluated 1218 schoolchildren aged 11–14 years in a well-defined territory of northern Italy. Diagnosis of VWD was considered "probable" in the following children: those with low VWF levels (VWF:RCo below an ABO-adjusted reference range) belonging to a family with more than two members, including or not the subject under investigation and those with a bleeding history consisting of two or more symptoms. A definite diagnosis was assigned if, in addition to these criteria, at least one other family member on the hemorrhagic side had a low VWF level. Ten children (four with probable and six with definite VWD) were classified as affected (0.82%). This figure could range from 7 (0.57%) to 14 (1.15%) taking into account the 90% confidence interval for the lower limit of the normal range. It turned out that all these children had at least a bleeding symptom. This translates into a prevalence of 0.57–1.15%, or 5700–11 500 per million. Interestingly, in about half of the diagnosed families from this investigation linkage was not subsequently confirmed [12].

In 1993, Werner *et al.* [23] published the results of a similar investigation carried out in 600 American schoolchildren aged 12–18 years undergoing well-child or school physical examinations at the pediatric ambulatory clinics of the hospitals located in Virginia, Ohio, and Mississippi. The criteria were seemingly less restrictive and included all three of at least one bleeding symptom, a family member with at least one bleeding symptom and low VWF. The overall prevalence was estimated at 1.3%, with no racial difference (1.15% among whites and 1.8% among blacks). These data have been confirmed in two additional studies, not reported as full papers. Miller *et al.* [24], in 1987, found a prevalence of VWD of 1.6% in adult blood donors from New York; however, the prevalence of symptomatic subjects with low VWF:RCo was 0.2%. In an additional study, Meriane *et al.* [25] studied the prevalence in Arabic–Turkish adult subjects. The figure was 1.23%, again with no racial differences. As a general comment, the prevalence of VWD appears to be similar in different ethnic groups. In all these studies the same functional test (VWF:RCo) and separate normal ranges according to blood groups were used, thus providing uniformity to the results. Even though these figures appear high, it should be emphasized that the prevalence is probably even higher since the sensitivity of the functional test is about 50%. This assumption stems from the demonstration by Miller *et al.* [26] that among the obligatory carriers for type 1 disease, only 42% had abnormal VWF activity on their initial test. As previously mentioned, most of these cases will resist a Bayesian approach as sufficiently proved VWD cases, in keeping with their lack of linkage with a VWF allele [12]. Of note, in a murine model a phenotype mimicking VWD was produced by a mutation in the glycosyltransferase (*GALgt2*) gene [27] and the occurrence of similar mutations in human "VWD" cannot presently be ruled out.

Table 43.3 Estimates of prevalence of von Willebrand disease (VWD).

VWD severity	Study	Methodology	Population	Prevalence
Severe	Weiss *et al.* [14]	Mail survey to 354 hematology departments	USA, Canada, 17 European countries, Iran, Israel	1.38–1.51 per million
	Mannucci *et al.* [15]	Patients identified through a questionnaire; plasma VWF assay and recruitment of patients with VWF:Ag < 1% by IRMA	Western European countries plus Israel	0.1–3.12 per million
	Berliner *et al.* [16]	Investigation of patients followed at a single center	Cases followed in Israel	5.3 per million among Arabs
Intermediate	Nilsson [17]	Cases registered at specialized centers in Sweden	230 Swedish families (530 patients) with VWD already known	70 cases per million inhabitants (about 15% severe type 3)
Mild	Rodeghiero *et al.* [2]	Anamnesis + VWF:RCo Family study	Caucasian children	0.82% (8200 per million)
	Rodeghiero *et al.* [22]	As above + VWF:Ag instead of VWF:RCo	As above	0.7%
	Miller *et al.* [24]	VWF:RCo	Adult blood donors	1.6% (0.2% bleeder)
	Meriane *et al.* [25]	Anamnesis + VWF:RCo Family study	Arabic–Turkish Adult students	1.23%
	Werner *et al.* [23]	Anamnesis + VWF:RCo Family study	Caucasian–black Children	1.3% (1.15% Caucasian, 1.81% black)

IRMA, ImmunoRadioMetric Assay; VWF, von Willebrand factor.

Frequency of von Willebrand disease subtypes

The relative frequency of subtypes of VWD has been estimated only from the series of single institutions. These are obviously flawed since they are based on severe and intermediate VWD patients (group A and B patients) only, indicative of a reporting bias. On epidemiologic grounds, the prevalence of mild VWD (group C patients), which is almost invariably due to type 1 VWD, appears to be 100–1000 times higher than that of the group A and B patients. Consequently, type 1 VWD in the population should be at least 100-fold more common than the other subtypes. Nevertheless, in referred patients there is a rather homogeneous distribution of VWD subtypes (Table 43.4) [16,17,28–31]. Overall, the data could be summarized for a relative percentage frequency of 70, 17, and 13 for type 1, 2, and 3 VWD in the 592 patients considered in these studies. These data are quite in accordance with an extensive, recent survey carried out by Federici *et al.* [32] among Italian hemophilia centers reporting a relative percentage frequency of 73, 21, and 6 for type 1, 2, and 3 VWD.

This general view has recently been challenged by the demonstration that several subtle abnormalities of multimeric pattern can be observed in patients previously diagnosed as type 1 VWD by using well-standardized laboratory methods [33]. However, from a practical point of view, these minor abnormalities do not prevent a complete biologic response to desmopressin, which is a good criterion for considering these patients to have a true quantitative deficiency of VWF [34].

Prevalence of von Willebrand disease in developing countries

Limited investigations have been carried out in developing countries, based on voluntary reporting in mail questionnaire-based survey through national or regional hemophilia centers [21]. In general, major under-reporting is evident compared with the expected prevalence. In a more recent unpublished survey, the ratio between severe hemophilia A (taken as a normalizing prevalence) and that of VWD in the same region was investigated, showing an underreporting of more than 60% of cases of VWD. Surprisingly, the distribution of severity in reported cases is apparently not much different from that reported in developed countries, indicating the lack of a regional or national strategy for the detection of more severe cases [35]. Sadly, deaths secondary to hemorrhage in VWD patients are still reported from these countries, despite cryoprecipitate and VWF/factor VIII concentrates being generally available, whereas similar cases are no longer described in economically more developed countries.

Table 43.4 Frequency of von Willebrand disease types in different series of patients.

Authors	Number of patients	Type 1 (%)	Type 2 (%)	Type 3 (%)
Tuddenham *et al.* [28]	134	75	19	6
Lenk *et al.* [29]	111	76	12	12
Nilsson [17]	106 families	70	10	20
Hoyer *et al.* [30]	116	71	23	6
Awidi [31]	65	59	29.5	11.5
Berliner *et al.* [16]	60	62	9	29
Overall	592	70	17	13

Practical implications

Severe VWD is a rare disorder, with a prevalence similar to that of other severe, homozygous coagulopathies, whereas the prevalence of intermediate VWD is probably similar to the cumulative prevalence of hemophilia A and B. The prevalence of mild VWD is still uncertain, but a reasonable estimate is possibly one case between 1000 and 10 000 subjects. About 1% of the normal population could satisfy quite conservative epidemiologic criteria sufficient to diagnose VWD in *ad hoc* cross-sectional investigations. This rather high prevalence figure must be interpreted with caution, as many of these subjects will experience only minor or trivial bleedings during their lifetime and will probably never be referred for medical assistance. It is therefore of paramount importance to distinguish between a diagnosis satisfying only minimal criteria and a clinically meaningful diagnosis. The first type of diagnosis is relevant for the epidemiologist but could be dangerous to the patients and his/her family in terms of generated anxiety and social burden; by contrast, the second one is certainly more useful for the patient and the physician alike [36,37]. We will discuss here two initiatives to clarifying this issue using an epidemiologic approach.

The case of presurgical screening

A prevalence of 1 per 500 subjects of a hemorrhagic disease may certainly be alarming for a surgeon facing an elective mucous surgery (e.g., tonsillectomy) who is keen to avoid any hemorrhagic risk. However, despite such a high prevalence in the population, a presurgical screening is probably not cost-effective for two reasons: First, all available phenotypic laboratory tests have their specificity based on the "reference limit" concept, which means that the specificity is usually set at 97.5%. Even assuming a sensitivity of 100% for a laboratory test (a very optimistic estimate indeed), for every 1000 subjects screened, the test would identify 25 subjects as affected, of whom 23 would be false positives and only two would have mild VWD. Second, the chance of patients with mild VWD bleeding significantly after surgery may be less than 50%, which means that more than 1000 subjects would need to be screened to avoid a surgical bleeding related to mild VWD, at the cost of excluding all the false positives.

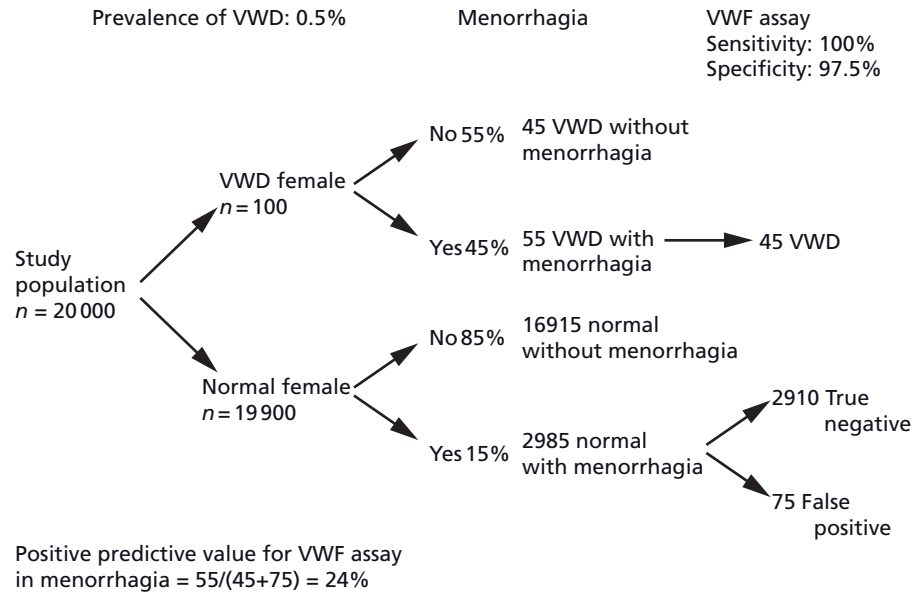

Figure 43.2 Positive predictive value for von Willebrand factor assay in women referred for menorrhagia. See text for assumptions. Study population n = 20 000.

The case of a diagnosis based on mild bleeding symptoms

Similarly, pursuing the diagnosis of mild VWD in a subject without a convincing personal or familial bleeding history poses the problems mentioned for presurgical screening and for cross-sectional epidemiologic surveys. As an example, we could examine the case of a woman investigated for VWD because of menorrhagia. Menorrhagia is found in 29–44% of otherwise healthy women [38,39] compared with 50–60% of VWD females [39]. Is screening for VWD advisable for all patients referred for menorrhagia? Let's consider a town with a population of 20 000 fertile women (Figure 43.2). Based on the prevalence of 0.5% of VWD and the above-mentioned figures, based on the frequency of menorrhagia in women with type 1 VWD [40] and in normal women [5], the ratio of VWD : normal in women with menorrhagia is 45 : 2985 in a population of 20 000 fertile women. By using the same laboratory test with a sensitivity of 100% and a specificity of 97.5% in all women complaining of menorrhagia, we can identify all the 45 women with VWD, but also 75 false positively "labeled" women. This represents a positive predictive value of only 37.5%.

Therefore, while it is recognized that menorrhagia could be the sole presenting symptom of a hemorrhagic disorder [41] and that in women with menorrhagia there is a high prevalence of women with VWD [42], a generalized screening is not advised. This is even truer if one considers that only a limited benefit is expected from a specific diagnosis in most women.

A clinically useful diagnosis

The two above-mentioned instances share a common feature: a laboratory diagnosis is made on the basis of a personal history with few symptoms (e.g., menorrhagia) or no symptoms at all (screening). In a recent multicenter survey, we demonstrated that the pretest probability (likelihood ratio) of VWD is significantly increased only when a clinically relevant history of hemorrhage is present (at least two hemorrhagic symptoms in the proband or a bleeding score >3 in males and >5 in females) [5]. Moreover, at least two (but preferably three) family members should be present in a family to reasonably suspect VWD in cases with mild VWF reduction or dubious bleeding symptoms [13]. Therefore, every laboratory assessment should be undertaken only in subjects presenting with significant bleeding symptoms or having two first-degree relatives with hemorrhagic symptoms or a VWF level < 40 IU/dL. It is also worth noting that the subjects and families identified by these criteria are also those that are more likely to benefit from an appropriate therapy (e.g., desmopressin treatment or prophylaxis).

These epidemiologic data further reaffirm the concept that physicians should always base their diagnoses on sound clinical criteria that will translate their specific diagnosis into a beneficial way to treat or prevent the consequences of this still intriguing disorder [36].

Acknowledgment

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References

1 von Willebrand EA. Hereditär pseudohemofili. *Finska Läkarsällskapets Handl* 1926; **67**: 7–112.

2 Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. *Blood* 1987; **69**: 454–9.
3 Higham JM, O'Brien PM, Shaw RW. Assessment of menstrual blood loss using a pictorial chart. *Br J Obstet Gynaecol* 1990; **97**: 734–9.
4 Friberg B, Orno AK, Lindgren A, Lethagen S. Bleeding disorders among young women: a population-based prevalence study. *Acta Obstet Gynecol Scand* 2006; **85**: 200–6.
5 Rodeghiero F, Castaman G, Tosetto A, *et al*. The discriminant power of bleeding history for the diagnosis of von Willebrand disease type 1: an international, multicenter study. *J Thromb Haemost* 2005; **3**: 2619–26.
6 Hedlund-Treutiger I, Revel-Vilk S, Blanchette VS, Curtin JA, Lillicrap D, Rand ML. Reliability and reproducibility of classification of children as "bleeders" versus "non-bleeders" using a questionnaire for significant mucocutaneous bleeding. *J Pediatr Hematol Oncol* 2004; **26**: 488–91.
7 Sadler JE, Budde U, Eikenboom JC, *et al*.; Working Party on von Willebrand Disease Classification. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. *J Thromb Haemost* 2006; **4**: 2103–14.
8 Castaman G, Federici AB, Rodeghiero F, Mannucci PM. Von Willebrand's disease in the year 2003: towards the complete identification of gene defects for correct diagnosis and treatment. *Haematologica* 2003; **88**: 94–108.
9 Goodeve A, Eikenboom J, Castaman G, *et al*. Phenotype and genotype of a cohort of families historically diagnosed with type 1 von Willebrand disease in the European study, Molecular and Clinical Markers for the Diagnosis and Management of Type 1 von Willebrand Disease (MCMDM-1VWD). *Blood* 2007; **109**: 112–21.
10 Eikenboom J, Van Marion V, Putter H, *et al*. Linkage analysis in families diagnosed with type 1 von Willebrand disease in the European study, molecular and clinical markers for the diagnosis and management of type 1 VWD. *J Thromb Haemost* 2006; **4**: 774–82.
11 James PD, Paterson AD, Notley C, *et al*.; Association of Hemophilia Clinic Directors of Canada. Genetic linkage and association analysis in type 1 von Willebrand disease: results from the Canadian type 1 VWD study. *J Thromb Haemost* 2006; **4**: 783–92.
12 Castaman G, Eikenboom JCJ, Bertina R, Rodeghiero F. Inconsistency of association between type 1 von Willebrand disease phenotype and genotype in families identified in an epidemiologic investigation. *Thromb Haemostas* 1999; **82**: 1065–70.
13 Tosetto A, Castaman G, Rodeghiero F. Evidence-based diagnosis of type 1 von Willebrand disease: a Bayes theorem approach. *Blood* 2008; **111**: 3998–4003.
14 Weiss HJ, Ball AP, Mannucci PM. Incidence of severe von Willebrand's disease. *N Engl J Med* 1982; **307**: 127.
15 Mannucci PM, Bloom AL, Larrieu MJ, Nilsson IM, West RR. Atherosclerosis and von Willebrand factor. I. Prevalence of severe von Willebrand's disease in Western Europe and Israel. *Br J Haematol* 1984; **57**: 163–9.
16 Berliner SA, Seligsohn U, Zivelin A, Zwang E, Sofferman G. A relatively high frequency of severe (type III) von Willebrand's disease in Israel. *Br J Haematol* 1986; **62**: 535–43.
17 Nilsson IM. Von Willebrand disease from 1926 to 1983. *Scand J Haematol* 1984; **33**(Suppl. 40): 21–43.
18 Bloom AL, Giddins JC. HIV infection and AIDS in von Willebrand's disease. An international survey including data on the prevalence of clinical von Willebrand's disease. In: Lusher JM, Kessler CM (eds.) *Hemophilia and von Willebrand's Disease in 1990s*. Amsterdam: Elsevier Science Publishers, 1991: 405–11.
19 Bloom AL. The von Willebrand syndrome. *Semin Hematol* 1980; **17**: 215–27.
20 Bachman F. Diagnostic approach to mild bleeding disorders. *Semin Hematol* 1980; **17**: 292–312.
21 Sadler JE, Mannucci PM, Berntorp E, *et al*. Impact, diagnosis and treatment of von Willebrand disease. *Thromb Haemost* 2000; **84**: 160–74.
22 Rodeghiero F, Castaman G, Tosetto A. von Willebrand factor antigen is less sensitive than ristocetin cofactor for the diagnosis of type I von Willebrand disease—results based on a epidemiological investigation. *Thromb Haemostas* 1990; **64**: 349–52.
23 Werner EJ, Broxson EH, Tucker EL, *et al*. Prevalence of von Willebrand disease in children: A multiethnic study. *J Pediat* 1993; **123**: 893–8.
24 Miller CH, Lenzi R, Breen C. Prevalence of von Willebrand's disease among U.S. adults. *Blood* 1987; **70**(Suppl. 1): 377 (Abstract).
25 Meriane F, Sultan Y, Arabi H, *et al*. Incidence of a low von Willebrand factor activity in a population of Algerian students. *Blood* 1991; **78**(Suppl. 1): 484 (Abstract).
26 Miller CH, Graham JB, Goldin LR, Elston RC. Genetics of classic von Willebrand's disease. I. Phenotypic variation within families. *Blood* 1979; **54**: 117–36.
27 Nichols WC, Cooney KA, Mohlke KL, *et al*. von Willebrand disease in the RIIIS/J inbred mouse strain as a model for von Willebrand disease. *Blood* 1994; **83**: 3225–8.
28 Tuddenham EGD. von Willebrand factor and its disorders: an overview of recent molecular studies. *Blood Rev* 1989; **3**: 251–62.
29 Lenk H, Nilsson IM, Holmberg L, Weissbach G. Frequency of different types of von Willebrand's disease. *Acta Med Scand* 1988; **224**: 275–80.
30 Hoyer LW, Rizza CR, Tuddenham EGD, *et al*. von Willebrand factor multimer patterns in von Willebrand's disease. *Br J Haematol* 1983; **55**: 493–507.
31 Awidi AS. A study of von Willebrand's disease in Jordan. *Ann Hematol* 1992; **64**: 299–302.
32 Federici AB, Castaman G, Mannucci PM. Guidelines for the diagnosis and treatment of von Willebrand disease in Italy. Italian Association of Hemophilia Centers (AICE). *Haemophilia* 2002; **8**: 607–21.
33 Budde U, Schneppenheim R, Eikenboom J, *et al*. Detailed von Willebrand factor multimer analysis in patients with von Willebrand disease in the European study, molecular and clinical markers for the diagnosis and management of type 1 von Willebrand disease (MCMDM-1VWD). *J Thromb Haemost* 2008; **6**: 762–71.
34 Castaman G, Lethagen S, Federici AB, *et al*. Response to desmopressin is influenced by the genotype and phenotype in type 1 von Willebrand disease (VWD): results from the European Study MCMDM-1VWD. *Blood* 2008; **111**: 3531–9.
35 Srivastava A, Rodeghiero F. Epidemiology of von Willebrand disease in developing countries. *Semin Thromb Hemost* 2005; **31**: 569–76.
36 Rodeghiero F. von Willebrand disease: still an intriguing disorder in the era of molecular medicine. *Haemophilia* 2002; **8**: 292–300.
37 Rodeghiero F, Castaman G. Congenital von Willebrand disease type 1: definition, phenotypes, clinical and laboratory assessment. In: Michiels JJ (ed.) von Willebrand Factor and von Willebrand disease. *Clin Haematol* 2001; **14**: 321.

38 Sramek A, Eikenboom JC, Briet E, Vandenbroucke JP, Rosendaal FR. Usefulness of patient interview in bleeding disorders. *Arch Intern Med* 1995; **155**: 1409–15.
39 Kadir RA, Economides DL, Sabin CA, Pollard D, Lee CA. Assessment of menstrual blood loss and gynaecological problems in patients with inherited bleeding disorders. *Haemophilia* 1999; **5**: 40–8.
40 Tosetto A, Rodeghiero F, Castaman G, *et al*. Impact of plasma von Willebrand factor levels in the diagnosis of type 1 von Willebrand disease: Results from a multicenter European study (MCMDM-1VWD). *J Thromb Haemost* 2006; **4**: 766–73.
41 Edlund M, Blomback M, von Schoultz B, Andersson O. On the value of menorrhagia as a predictor for coagulation disorders. *Am J Hematol* 1996; **53**: 234–8.
42 Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. Frequency of inherited bleeding disorders in women with menorrhagia. *Lancet* 1998; **351**: 485–9.

44 von Willebrand disease: biologic diagnosis

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Diagnosis of von Willebrand disease (VWD) requires that the patient have both a personal and family history of bleeding symptoms as well as laboratory findings consistent with the diagnosis [1,2]. Laboratory testing is the most quantifiable of these criteria, but may occasionally lead to erroneous diagnosis. Precise cut-off values for diagnosis of both type 1 and type 2M VWD are in fact the subject of much current debate. On the other hand, no standard tools exist to quantitate personal or familial bleeding symptoms outside of research questionnaires, although such tools have been recently developed [3,4]. Pediatric patients, in particular, may not yet have experienced significant hemostatic challenges and therefore may have fewer bleeding complaints than adults with similar VWF levels. Thus, despite its limitations, laboratory testing for VWD remains a crucial part of the coagulation work-up for patients with bleeding symptoms.

von Willebrand factor screening tests

While a significant personal and family history of bleeding symptoms may lead to the suspicion of VWD, laboratory tests are required to confirm this diagnosis. Screening tests for VWD are imprecise and normal screening tests do not necessarily preclude the diagnosis of VWD in a given patient. However, these tests are easily performed by community practitioners and may help determine which patients require a hematology referral. The abbreviations for common VWF-related laboratory tests are listed in Table 44.1. Among the screening tests commonly performed in diagnosing bleeding disorders, the partial thromboplastin time (PTT), bleeding time, and, more recently, the platelet function analyzer, or PFA-100, are commonly considered to screen for the presence of VWD.

Complete blood count

Although it is not technically a screening test, a complete blood count (CBC) is often obtained as part of the work-up for patients with potential bleeding disorders. In VWD, the CBC is usually normal, although patients with significant bleeding, especially women with menorrhagia, may present with anemia. Type 2B VWD is often associated with thrombocytopenia, as is platelet-type, pseudo-VWD owing to clearance of VWF–platelet complexes from circulation. Thrombocytopenia, however, is not invariably present in type 2B VWD, limiting use of the CBC as a screening test [5].

Partial thromboplastin time

The PTT is frequently prolonged slightly in patients with VWD due to the concomitant decrease in factor VIII (FVIII) in the presence of low VWF levels, particularly in type 2N and type 3 VWD. A prolonged PTT is not invariably present, however, and even a normal PTT does not exclude mild VWD. The normal range for the PTT varies by laboratory, so that values obtained must be compared to a reference standard. The potential coexistence of low VWF levels and factor XII deficiency, previously referred to as VWD San Diego [6], suggests that a significant prolongation in the PTT accompanied by borderline normal FVIII and VWF levels may be the result of mild factor XII deficiency instead of, or in addition to, VWD.

Bleeding time

The Ivy bleeding time involves the application of a small blade to perform a standardized skin incision, while at the same time a blood pressure cuff is utilized to maintain a standard blood pressure at the site of injury [7]. This test is neither sensitive nor specific for the presence of low VWF levels, with a sensitivity of around 30% [8,9]. Operator skill and patient cooperation may also affect interpretation of the bleeding time results, and consequently this test is no longer recommended, especially in children.

Platelet function analyzer

The platelet function analyzer, or PFA-100, is another method of screening platelet function in coagulation. This test utilizes membranes impregnated with platelet agonists, usually

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Table 44.1 Abbreviations for common laboratory tests.

CBC	Complete blood count
PTT	Partial thromboplastin time
PFA	Platelet function analyzer
VWF:Ag	VWF antigen
VWF:RCo	VWF ristocetin cofactor activity
FVIII:C	Factor VIII activity
VWF:CB	VWF–collagen binding
VWFpp	VWF propeptide
LD-RIPA	Low-dose ristocetin-induced platelet aggregation
VWF:PB	VWF–platelet binding
VWF:F8B	VWF–FVIII binding

VWF, von Willebrand factor.

collagen and epinephrine or collagen and adenosine diphosphate (ADP), through which a whole blood sample from the patient is aspirated and the time to membrane occlusion measured [10]. The use of whole blood under shear conditions is intended to mimic *in vivo* conditions. Although currently widely used, the sensitivity of this test has been subject to considerable debate. Initial reports suggested that the sensitivity was close to 100% [11,12], while subsequent studies have reported sensitivities ranging from 30% to 80% when different populations are screened [8,9]. A prolonged PFA does not necessarily mean a diagnosis of VWD, as this test will also be abnormal in platelet function disorders, and a normal PFA does not necessarily exclude the diagnosis of VWD. This lack of sensitivity and specificity prevents the PFA-100 from being used as a definitive VWD screening test.

von Willebrand factor diagnostic tests

Because no reliable screening tests are available, definitive diagnosis of VWD relies on specific assays of VWF function along with the clinical history. The VWF antigen (VWF:Ag) measures the total amount of VWF protein present in plasma. The VWF ristocetin cofactor assay (VWF:RCo) measures VWF activity as a function of its ability to agglutinate platelets in the presence of ristocetin. VWF multimer structure, in combination with VWF:Ag, VWF:RCo, and FVIII:C, may be offered as part of a "VWD panel." Available testing is summarized in Table 44.2.

As always with coagulation testing, sample handling and the quality of the reference laboratory is important, and laboratory findings must be interpreted in the context of the clinical history. Careful attention to the reference standard is required, particularly when results are expressed in international units (IU). The World Health Organization (WHO) international standard is recommended for calibration so that results may be compared across laboratories.

Table 44.2 Summary of von Willebrand factor (VWF) laboratory testing.

Screening tests	CBC
	PTT
	Bleeding time[a]
	PFA-100[a]
Diagnostic tests	VWF:Ag
	VWF:RCo
	FVIII:C
	VWF multimers
Confirmatory tests	VWF:CB
	VWFpp
	VWF gene sequencing
	LD-RIPA
	VWF:PB
	VWF:F8B

[a]Not routinely recommended.

VWF:Ag

The concentration of VWF:Ag is quantified by immunoassays, either by enzyme-linked immunosorbent assay (ELISA) or by automated latex immunoassay (LIA) [13]. While these assays reliably detect quantitative deficiencies of VWF, further testing is required to distinguish the qualitative variants since the VWF:Ag does not depend on the presence of functional protein. Because VWF:Ag levels are influenced by a number of factors, a single normal value does not reliably rule out the diagnosis of VWD. Since most laboratories establish the normal range as the (log-transformed) mean ± 2 SD, normal individuals may account for 2.5% of the population below the normal range, creating a problem with VWD overdiagnosis [14].

VWF:Ag levels vary widely in the general population, with total VWF protein in plasma reported as 50–240 IU/dL. Blood type accounts for a significant percentage of VWF variation, with mean VWF:Ag approximately 25–30% lower in patients with blood group O [15]. Type O is certainly over-represented in mild VWD, and some laboratories report normal VWF levels for each blood group, but this may not be relevant given that symptomatic bleeding seems to correlate with VWF level, rather than blood type per se. VWF levels are also increased in African Americans [16]. Age, stress, pregnancy, trauma, and many other underlying inflammatory states have been associated with an elevation in VWF levels as well and may hinder the diagnosis of VWD [17].

VWF:RCo

von Willebrand factor activity is most often measured by the ristocetin cofactor activity, or VWF:RCo. This assay exploits the ability of the antibiotic ristocetin to induce a conformational change in VWF that then enables VWF to bind platelet

GPIb [18]. Because ristocetin preferentially induces binding of high-molecular-weight VWF multimers, it is also a surrogate test for high-molecular-weight multimer presence and functionality. A discrepancy between VWF:Ag and VWF:RCo is the hallmark of type 2 VWD, particularly the 2A, 2B, and 2M variants [19]. Most VWF:RCo assays are performed using platelet aggregometry or turbidometry, with normal platelets serving as the GPIb source, and patient plasma as the VWF source. Reproducibility is a major problem with the VWF:RCo assay, however, as is the variability from laboratory to laboratory [20,21]. The possibility of an ELISA-based assay may improve standardization of the VWF:RCo in the future [22]. Flow-cytometry based versions of this assay are also under development [23].

FVIII:C

Factor VIII activity is also useful in VWF diagnosis. Most laboratories use a one-stage FVIII activity assay to quantitate the amount of FVIII present. In most type 1 VWD patients, FVIII:C is either similar to or slightly higher than the VWF:Ag [2]. In type 2N, however, the reduced binding of FVIII to VWF results in disproportionately low FVIII levels compared with VWF levels. Type 3 VWD patients will have extremely low FVIII:C, typically <10 IU/dL. Since FVIII is usually detectable in plasma of patients with type 3 VWD, this represents the low steady-state level in the absence of stabilization by VWF.

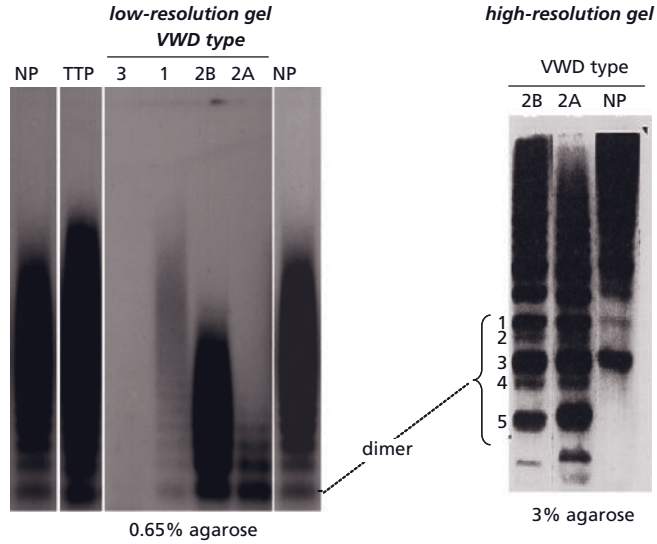

Figure 44.1 von Willebrand factor (VWF) multimers. On the left, a low-resolution gel shows multimers for a normal plasma sample (NP), a patient with thrombotic thrombocytopenic purpura (TTP) with larger than normal multimers, and four von Willebrand disease (VWD) subtypes—type 3, type 1, type 2B, and type 2A. On the right, a high-resolution gel shows separation of the lowest, dimer, band into five distinct bands, but lacks the ability to demonstrate loss of high-molecular-weight multimers in type 2B and 2A VWD. Used by permission of R.R. Montgomery.

VWF multimers

Multimer analysis is crucial for detection of several of the type 2 VWD variants. Agarose gel electrophoresis of a plasma sample enables visualization of multimers of all sizes [24]. The procedure is technically difficult, however, and not all laboratories are able to produce gels of sufficient quality to allow precise interpretation. Recently, subtle differences in multimer pattern have been linked to certain VWF subtypes, as demonstrated by the sensitive multimer gels performed by Budde and colleagues [25]. In type 1 VWD, multimers of all sizes will be present, albeit at lower concentrations than normal, while multimers are usually absent in type 3 VWD. Multimer analysis is most useful in diagnosis of type 2A and 2B VWD, which present with absence of the high-molecular-weight multimers (Figure 44.1). Cardiac defects may also contribute to loss of high-molecular-weight multimers [26]. ADAMTS13 deficiency, either congenital or acquired, as in thrombotic thrombocytopenic purpura patients, may demonstrate a pattern of ultralarge multimers owing to the lack of cleavage following VWF release into plasma.

von Willebrand factor confirmatory tests

The quantitative VWD variants, type 1 and type 3, are diagnosed based on low or absent VWF:Ag and VWF:RCo. The qualitative, type 2 variants often require confirmatory testing in order to make a definitive diagnosis. Currently, testing is available for three functional aspects of VWF: collagen binding (VWF:CB), platelet binding (VWF:PB), and factor VIII binding (VWF:F8B). VWF:PB is helpful in differentiating type 2B VWD from platelet-type, pseudo-VWD, along with low-dose ristocetin-induced platelet aggregation (LD-RIPA). VWF:F8B is used in diagnosis of type 2N VWD and reflects the reduced affinity of VWF for FVIII in type 2N patients. There is no confirmatory test for type 2M VWD at present, other than the presence of a decreased VWF:RCo/VWF:Ag ratio in the presence of normal VWF multimers.

VWF:CB

Collagen binding is another measure of VWF function. The collagen binding assay, or VWF:CB, measures the capacity of plasma VWF to bind collagen [27,28]. This assay is also ELISA-based, but the type of collagen used is critical. Human type 3 collagen is the most sensitive, but the use of a combination of type 1 and type 3 collagen has been advocated as providing more specificity [29]. Although VWF:CB has not replaced VWF:RCo in the work-up of VWD, it may offer additional information regarding VWF function. Mutations in the A3 domain of VWF may only be detected by demonstration of a low VWF:CB [30].

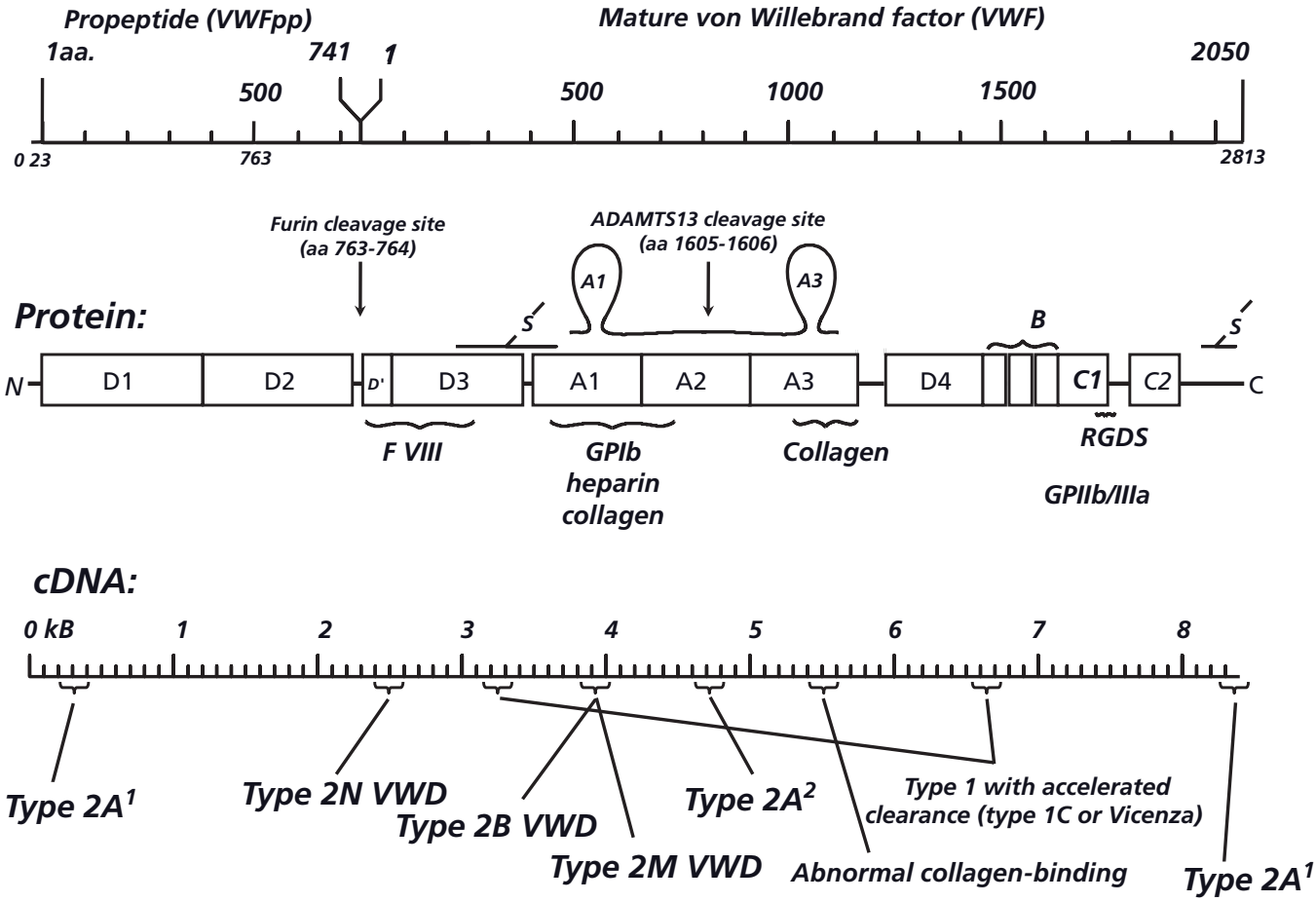

Figure 44.2 von Willebrand factor (VWF) cDNA and protein domains with sites of common genetic mutations. The VWF protein is diagrammed in the top panel. The middle panel shows the various functional domains of the VWF protein. The bottom panel displays the cDNA with locations of common VWF mutations for many of the von Willebrand disease (VWD) subtypes. The type 2A[1] mutations are characterized by abnormal multimer synthesis, while the 2A[2] mutations demonstrate increased proteolysis. Used by permission of R.R. Montgomery.

VWFpp

The VWF propolypeptide, VWFpp, is secreted from cells in equimolar concentration to mature VWF [31]. Some VWD patients have increased clearance of VWF from the circulation, and these patients are characterized by high propeptide levels and a short half-life of VWF following administration of desmopressin. An abnormal VWFpp/VWF:Ag ratio therefore is consistent with increased clearance, with a proposed classification of VWD 1C [32]. This category would also include the well-recognized Vicenza variant [33,34]. VWFpp levels may be helpful in distinguishing patients with clearance defects from those with suboptimal VWF expression. In those with accelerated clearance of VWF, FVIII levels are also reduced through this accelerated clearance [32]. VWFpp is also decreased in type 2A and type 2B VWD [35].

VWF gene sequencing

Genetic testing is also becoming more widely available. DNA analysis of VWF is challenging because of the large size of the VWF protein and complexity of the genomic DNA, with 52 exons and approximately 8.4 kb of coding sequence [36]. Type 2 and type 3 VWD are more likely to be associated with gene defects, while type 1 VWD patients, especially those with VWF:Ag > 20 IU/dL, most often do not have an associated mutation in the VWF gene, especially if normal multimers are present [37,38]. The majority of type 2A, 2B, and 2M mutations are located in exon 28, although some type 2A mutations have been found in other regions, including exons 11–16, 26, 51, and 52. While type 2N defects are most often seen in exon 18, mutations affecting FVIII binding have also been reported in exons 17–27. Type 1 defects are scattered throughout the gene. The locations of some of the common VWD mutations are shown in Figure 44.2. The mutations and polymorphisms in the VWF gene are detailed in an online database (http://www.vwf.group.shef.ac.uk). There are numerous naturally occurring polymorphisms as well, making it difficult to use gene sequencing for definitive diagnosis of type 1 VWD patients. Those type 1 patients who do not have a documented mutation may have a splice site

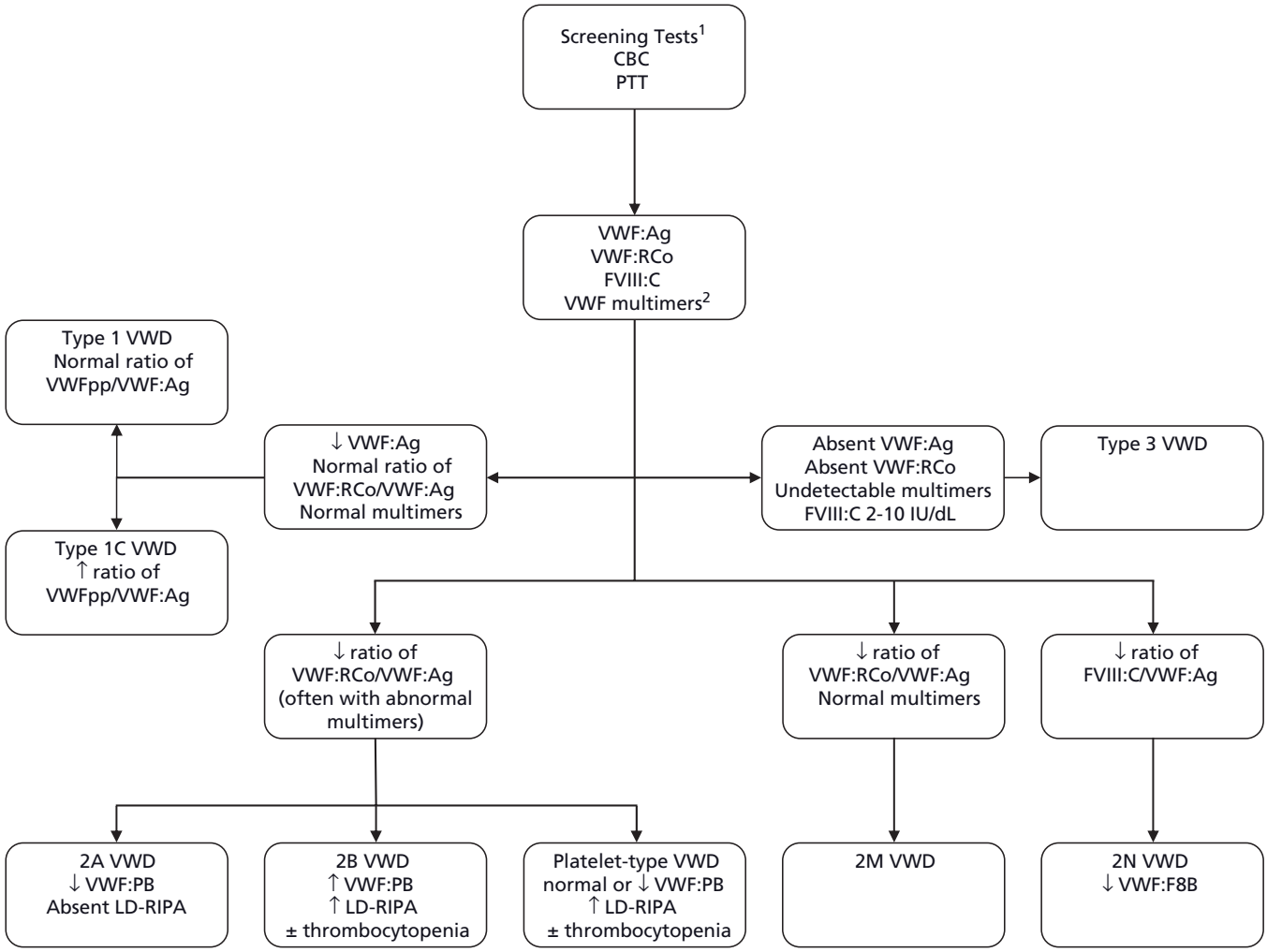

Figure 44.3 Laboratory testing algorithm for von Willebrand disease.
[1]Additional screening tests may be indicated depending on symptoms and other diagnoses under consideration.
[2]Particularly if ↓VWF:Ag or ↓VWF:RCo/VWF:Ag.

mutation, promoter defect, or a defect in a related gene that causes low VWF levels. The possibility also exists that such patients simply have low VWF levels and do not truly have hereditary VWD.

Low-dose ristocetin-induced platelet aggregation

Type 2B VWD is a gain-of-function variant, with increased binding to platelet GPIb. Low-dose ristocetin (typically <0.6 mg/mL) is added to platelet-rich plasma and monitored for aggregation, which should only occur in type 2B VWD or in patients with platelet GPIb mutations that also enable spontaneous VWF binding, referred to as platelet-type, pseudo-VWD [2].

VWF:PB

Direct platelet binding assays are used to confirm the diagnosis of type 2B VWD. In this assay, fixed platelets are incubated with plasma and low-dose ristocetin (typically <0.6 mg/mL). A neutral antibody is used to detect VWF that binds spontaneously to the normal platelets [39]. This test will confirm that the gain-of-function defect is due to plasma VWF, and exclude the possibility of platelet-type, pseudo-VWD.

VWF:F8B

In type 2N VWD, the defect is in the ability of VWF to bind FVIII. FVIII binding assays are also ELISA based, and utilize an antibody to isolate plasma VWF from the patient [40,41]. Recombinant FVIII is added, its presence detected by antibody or activity assays, and compared with the amount of VWF:Ag. The ratio of FVIII to VWF will be decreased in type 2N VWD, in contrast to normal individuals. The FVIII/VWF is also decreased in mild hemophilia A, but there normal VWF–FVIII binding is present. In addition, type 2N VWD patients typically have a heightened FVIII response to DDAVP administra-

	Normal	Type 1	Type 1C (Vicenza)	Type 3	Type 2A	Type 2B	Type 2N	Type 2M	PT-VWD
VWF:Ag	N	↓	↓↓	absent	↓	↓	N or ↓	↓ or N	↓
VWF:RCo	N	↓	↓↓	absent	↓↓↓	↓↓	N or ↓	↓↓	↓↓
FVIII:C	N	N or ↓	↓	2–10 IU/dL	N or ↓	N or ↓	↓↓	N	N or ↓
VWFpp/VWF:Ag ratio	N	N	↑↑	absent	N or ↑	↑	N	N	↑
RIPA	N	often N	↓	absent	↓	often N	N	N or ↓	often N
LD-RIPA	absent	absent	absent	absent	absent	↑↑↑	absent	absent	↑↑↑
PFA*	N	N or ↑	↑	↑↑↑	↑	↑	N	↑	↑
BT*	N	N or ↑	↑	↑↑↑	↑	↑	N	↑	↑
Platelet count	N	N	N	N	N	↓ or N	N	N	↓
VWF multimers	N	N but ↓	N but ↓	absent	abnormal	abnormal	N but ↓	N but ↓	abnormal

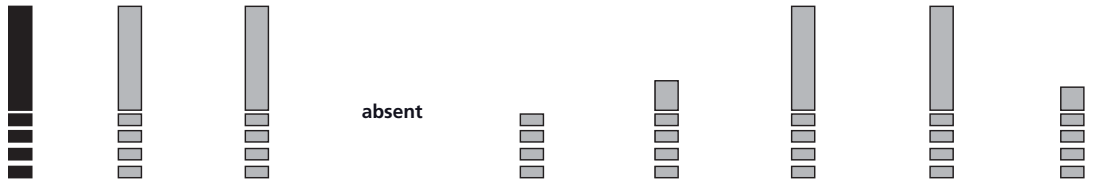

Figure 44.4 Expected results of von Willebrand disease (VWD) laboratory testing. PT-VWD, platelet-type VWD; N, normal; ↓,↓↓,↓↓↓, relative decrease; ↑, ↑↑, ↑↑↑, relative increase; N but ↓, normal but decreased in intensity. Used by permission of R.R. Montgomery.
*Not routinely recommended.

tion, but a shortened FVIII survival owing to the lack of normal FVIII–VWF binding. This assay may help to distinguish 2N VWD from mild factor VIII deficiency [42].

von Willebrand disease diagnosis

If there is clinical suspicion for the diagnosis of VWD, specific VWF testing should be performed as there are no good screening tests for this condition. Recommended initial testing includes VWF:Ag, VWF:RCo, FVIII:C, and multimer analysis [2]. A discrepancy between VWF:Ag and VWF:RCo should prompt further testing. A testing algorithm for the work-up of a potential VWD patient is shown in Figure 44.3. If FVIII is low, consideration of type 2N should occur, in which case VWF:F8B or DNA sequencing of the commonly affected exons may be helpful. Thrombocytopenia is frequently (although not always) seen with type 2B, and may prompt either ristocetin-induced platelet agglutination (RIPA), platelet-binding assay, or exon 28 sequencing. While blood group typing is performed with VWD studies at some institutions, the argument that low VWF levels, regardless of blood type, may cause bleeding makes this a less critical part of the laboratory evaluation. DNA sequencing may also be helpful in some of the type 2 variants. Expected results of VWD laboratory testing for the various VWD subtypes are shown in Figure 44.4.

Limitations of the currently available testing include the high variability present in the VWF:RCo and the need for more physiologic assays of VWF function. VWF *in vivo* is active under conditions of high shear forces, yet none of the readily available diagnostic tests specifically evaluates VWF under shear conditions. Also, laboratory testing provides an incomplete picture without adding in bleeding symptoms and family history. Recent efforts to generate predictive models have incorporated laboratory data from family members as part of the work-up for VWD [43]. Although family testing is not an obligatory part of the VWD work-up, it may provide additional diagnostic information. The laboratory results should always be correlated with clinical symptoms for the most accurate diagnosis of VWD and optimal patient care. In patients with low VWF levels who do not meet the criteria for diagnosis of VWD, treatment may depend on symptoms and clinical history, consistent with the concept of low VWF levels as a risk factor for bleeding.

References

1 Sadler JE, Rodeghiero F, ISTH SSC Subcommittee on von Willebrand Factor. Provisional criteria for the diagnosis of VWD type 1. *J Thromb Haemost* 2005; **3**: 775–7.

2 Nichols WL, Hultin MB, James AH, *et al.* Von Willebrand disease (VWD): Evidence-based diagnosis and management guidelines, the national heart, lung, and blood institute (NHLBI) expert panel report (USA). *Haemophilia* 2008; **14**: 171–232.

3 Rodeghiero F, Castaman G, Tosetto A, *et al.* The discriminant power of bleeding history for the diagnosis of type 1 von willebrand disease: an international, multicenter study. *J Thromb Haemost* 2005; **3**: 2619–26.

4 Tosetto A, Rodeghiero F, Castaman G, *et al.* A quantitative analysis of bleeding symptoms in type 1 von willebrand disease: Results from a multicenter European study (MCMDM-1 VWD). *J Thromb Haemost* 2006; **4**: 766–73.

5 Federici AB, Mannucci PM, Castaman G, *et al.* Clinical and molecular predictors of thrombocytopenia and risk of bleeding in patients with von willebrand disease type 2B: A cohort study of 67 patients. *Blood* 2009; **113**: 526–34.

6 Buchanan GR, Green DM, Handin RI. Combined von Willebrand disease and Hageman factor deficiency. *J Pediatr* 1977; **90**: 779–81.

7 Nilsson IM, Magnusson S, Borchgrevink C. The duke and ivy methods for determination of the bleeding time. *Thromb Diath Haemorrh* 1963; **10**: 223–34.

8 Dean JA, Blanchette VS, Carcao MD, *et al.* von Willebrand disease in a pediatric-based population—comparison of type 1 diagnostic criteria and use of the PFA-100 and a von Willebrand factor/collagen-binding assay. *Thromb Haemost* 2000; **84**: 401–9.

9 Quiroga T, Goycoolea M, Munoz B, *et al.* Template bleeding time and PFA-100 have low sensitivity to screen patients with hereditary mucocutaneous hemorrhages: comparative study in 148 patients. *J Thromb Haemost* 2004; **2**: 892–8.

10 Kundu SK, Heilmann EJ, Sio R, Garcia C, Davidson RM, Ostgaard RA. Description of an *in vitro* platelet function analyzer—PFA-100. *Semin Thromb Hemost* 1995; **21**(Suppl. 2): 106–12.

11 Fressinaud E, Veyradier A, Truchaud F, *et al.* Screening for von Willebrand disease with a new analyzer using high shear stress: a study of 60 cases. *Blood* 1998; **91**: 1325–31.

12 Cattaneo M, Lecchi A, Agati B, Lombardi R, Zighetti ML. Evaluation of platelet function with the PFA-100 system in patients with congenital defects of platelet secretion. *Thromb Res* 1999; **96**: 213–17.

13 Ingerslev J. A sensitive ELISA for von Willebrand factor (vWf:Ag). *Scand J Clin Lab Invest* 1987; **47**: 143–9.

14 Sadler JE. von Willebrand disease type 1: a diagnosis in search of a disease. *Blood* 2003; **101**: 2089–93.

15 Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ, Jr., Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood* 1987; **69**: 1691–5.

16 Miller CH, Haff E, Platt SJ, Rawlins P, Drews CD, Dilley AB, Evatt B. Measurement of von Willebrand factor activity: relative effects of ABO blood type and race. *J Thromb Haemost* 2003; **1**: 2191–7.

17 Pottinger BE, Read RC, Paleolog EM, Higgins PG, Pearson JD. von Willebrand factor is an acute phase reactant in man. *Thromb Res* 1989; **53**: 387–94.

18 Howard MA, Sawers RJ, Firkin BG. Ristocetin: a means of differentiating von Willebrand's disease into two groups. *Blood* 1973; **41**: 687–90.

19 Federici AB. Diagnosis of von Willebrand disease. *Haemophilia* 1998; **4**: 654–60.

20 Kitchen S, Jennings I, Woods TA, Kitchen DP, Walker ID, Preston FE. Laboratory tests for measurement of von Willebrand factor show poor agreement among different centers: results from the united kingdom national external quality assessment scheme for blood coagulation. *Semin Thromb Hemost* 2006; **32**: 492–8.

21 Meijer P, Haverkate F. An external quality assessment program for von Willebrand factor laboratory analysis: an overview from the European Concerted Action on Thrombosis and Disabilities Foundation. *Semin Thromb Hemost* 2006; **32**: 485–91.

22 Murdock PJ, Woodhams BJ, Matthews KB, Pasi KJ, Goodall AH. von Willebrand factor activity detected in a monoclonal antibody-based ELISA: an alternative to the ristocetin cofactor platelet agglutination assay for diagnostic use. *Thromb Haemost* 1997; **78**: 1272–7.

23 Chen D, Daigh CA, Hendricksen JI, Pruthi RK, Nichols WL, Heit JA, Owen WG. A highly-sensitive plasma von Willebrand factor ristocetin cofactor (VWF:RCo) activity assay by flow cytometry. *J Thromb Haemost* 2008; **6**: 323–30.

24 Ruggeri ZM, Zimmerman TS. Variant von Willebrand's disease: characterization of two subtypes by analysis of multimeric composition of factor VIII/von Willebrand factor in plasma and platelets. *J Clin Invest* 1980; **65**: 1318–25.

25 Budde U, Pieconka A, Will K, Schneppenheim R. Laboratory testing for von Willebrand disease: contribution of multimer analysis to diagnosis and classification. *Semin Thromb Hemost* 2006; **32**: 514–21.

26 Gill JC, Wilson AD, Endres-Brooks J, Montgomery RR. Loss of the largest von Willebrand factor multimers from the plasma of patients with congenital cardiac defects. *Blood* 1986; **67**: 758–61.

27 Brown JE, Bosak JO. An ELISA test for the binding of von Willebrand antigen to collagen. *Thromb Res* 1986; **43**: 303–11.

28 Favaloro EJ, Grispo L, Exner T, Koutts J. Development of a simple collagen based ELISA assay aids in the diagnosis of, and permits sensitive discrimination between type I and type II, von Willebrand's disease. *Blood Coagul Fibrinolysis* 1991; **2**: 285–91.

29 Favaloro EJ. Collagen binding assay for von willebrand factor (VWF:CBA): detection of von Willebrand's disease (VWD), and discrimination of VWD subtypes, depends on collagen source. *Thromb Haemost* 2000; **83**: 127–35.

30 Ribba AS, Loisel I, Lavergne JM, *et al.* Ser968Thr mutation within the A3 domain of von Willebrand factor (VWF) in two related patients leads to a defective binding of VWF to collagen. *Thromb Haemost* 2001; **86**: 848–54.

31 Borchiellini A, Fijnvandraat K, ten Cate JW, *et al.* Quantitative analysis of von Willebrand factor propeptide release *in vivo*: effect of experimental endotoxemia and administration of 1-deamino-8-D-arginine vasopressin in humans. *Blood* 1996; **88**: 2951–8.

32 Haberichter SL, Balistreri M, Christopherson P, *et al.* Assay of the von Willebrand factor (VWF) propeptide to identify type 1 von Willebrand disease patients with decreased VWF survival. *Blood* 2006; **108**: 3344–51.

33 Mannucci PM, Lombardi R, Castaman G, *et al.* Von Willebrand disease "Vicenza" with larger-than-normal (supranormal) von Willebrand factor multimers. *Blood* 1988; **71**: 65–70.

34 Casonato A, Pontara E, Sartorello F, *et al.* Reduced von Willebrand factor survival in type vicenza von Willebrand disease. *Blood* 2002; **99**: 180–4.

35 McCarroll DR, Levin EG, Montgomery RR. Endothelial cell synthesis of von Willebrand antigen II, von Willebrand factor, and

von Willebrand factor/von Willebrand antigen II complex. *J Clin Invest* 1985; **75**: 1089–95.

36 Mancuso DJ, Tuley EA, Westfield LA, *et al.* Structure of the gene for human von Willebrand factor. *J Biol Chem* 1989; **264**: 19514–27.

37 Goodeve A, Eikenboom J, Castaman G, *et al.* Phenotype and genotype of a cohort of families historically diagnosed with type 1 von Willebrand disease in the European study, molecular and clinical markers for the diagnosis and management of type 1 von Willebrand disease (MCMDM-1VWD). *Blood* 2007; **109**: 112–21.

38 James PD, Notley C, Hegadorn C, *et al.* The mutational spectrum of type 1 von Willebrand disease: results from a Canadian cohort study. *Blood* 2007; **109**: 145–54.

39 Scott JP, Montgomery RR. The rapid differentiation of type IIb von Willebrand's disease from platelet-type (pseudo-)von Willebrand's disease by the "neutral" monoclonal antibody binding assay. *Am J Clin Pathol* 1991; **96**: 723–8.

40 Montgomery RR, Hathaway WE, Johnson J, Jacobson L, Muntean W. A variant of von Willebrand's disease with abnormal expression of factor VIII procoagulant activity. *Blood* 1982; **60**: 201–7.

41 Mazurier C, Meyer D. Factor VIII binding assay of von Willebrand factor and the diagnosis of type 2N von Willebrand disease—results of an international survey on behalf of the subcommittee on von Willebrand factor of the scientific and standardization committee of the ISTH. *Thromb Haemost* 1996; **76**: 270–4.

42 Mazurier C, Gaucher C, Jorieux S, Goudemand M. Biological effect of desmopressin in eight patients with type 2N ("Normandy") von Willebrand disease. Collaborative group. *Br J Haematol* 1994; **88**: 849–54.

43 Tosetto A, Castaman G, Rodeghiero F. Evidence-based diagnosis of type 1 von Willebrand disease: a Bayes theorem approach. *Blood* 2008; **111**: 3998–4003.

45 Classification and clinical aspects of von Willebrand disease

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von Willebrand disease (VWD) is the most common inherited bleeding disorder and is caused by a deficiency and/or abnormality of von Willebrand factor (VWF), the high-molecular-weight glycoprotein that plays a major role in early phases of hemostasis [1]. VWD is inherited by autosomal dominant or recessive pattern, but women with milder VWD forms are apparently more symptomatic. VWD is also a very heterogeneous disorder and therefore patients with mild VWD forms are sometimes under- and misdiagnosed because of physiologic changes of VWF within the same individual and to the relative high variability of diagnostic tests [2–4].

Prevalence and classification

In population-based studies, the prevalence of VWD is very high (0.81%) [5], but the clinical relevance of many of these cases is uncertain. However, if we consider patients referred for clinical manifestations of bleeding, the actual prevalence is 66–100 cases per million of the general population [2–4]. The most updated classification of VWD has proposed six different types: VWD1, VWD3, VWD2A, VWD2B, VWD2M, and VWD2N [6]. A partial quantitative defect marks VWD1, whereas VWD3 is characterized by the nearly total absence of VWF in plasma and platelets. VWD1 is easily distinguished from VWD3 by milder VWF deficiency (usually in the range of 10–30 U/dL), an autosomal dominant pattern of inheritance and the presence of a milder bleeding tendency [6].

In the past, VWD1 was reported to be the most frequent form of VWD, accounting for approximately 70% of cases. A study based on the reappraisal of diagnoses of VWD1 after 10 years (1998–2008) in 1234 patients followed by 16 Italian centers has established that VWD1 represents only 671/1234 (55%) [7], because many cases previously diagnosed as VWD1 were re-diagnosed as VWD2. This change in classification was made because of discrepant VWF measurements [ratio of ristocetin cofactor activity (VWF:RCo) to VWF:Ag < 0.6] upon re-testing [2].

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Age and VWD type distribution of the 1234 patients enrolled into the Italian registry on VWD are shown in Figure 45.1a and b. The presence of qualitative defects of VWF in previously diagnosed VWD1 has been also reported in 154 families evaluated prospectively by the European study [8]. With regards to qualitative defects of VWF, four types are identified, reflecting different pathophysiologic mechanisms [6]. VWD2A and VWD2B are marked by the absence of high-molecular-weight VWF multimers in plasma but in VWD2B there is also an increased affinity of VWF for its platelet receptor, the glycoprotein Ib α (GpIbα). Characterization of qualitatively abnormal variants with decreased platelet-dependent function and a normal multimeric structure defines VWD2M. The VWD2N phenotype displays a full array of multimers, the defect being in the N-terminal region of the VWF where the binding domain for FVIII is located. This type is phenotypically distinguishable from mild hemophilia A only by the abnormal binding of FVIII to VWF (VWF:FVIIIB).

Clinical and laboratory diagnosis

Three main criteria are required for correct diagnosis of VWD: (i) positive bleeding history since childhood; (ii) reduced VWF activity in plasma; and (iii) history of bleeding in the family with autosomal dominant or recessive inheritance. Evidence-based diagnosis of VWD1 has been recently proposed according to these three criteria as published [9]. Clinical manifestations of VWD are excessive mucocutaneous bleeding and prolonged oozing after surgical procedures. In women, menorrhagia may be the only clinical manifestation. Soft-tissue and joint bleeding are rare, except in patients with VWD3, characterized by severe deficiencies of VWF and FVIII.

The clinical expression of the disease is usually mild in most patients with VWD1, whereas severity is typically greater in VWD2 and particularly in VWD3. Generally, the severity of bleeding correlates with the degree of reduction of VWF:RCo and FVIII. To date, only a few detailed descriptions of symptoms are available [2,10,11]. Table 45.1 shows the relative frequency of bleeding symptoms in three large series of patients diagnosed at specialized centers.

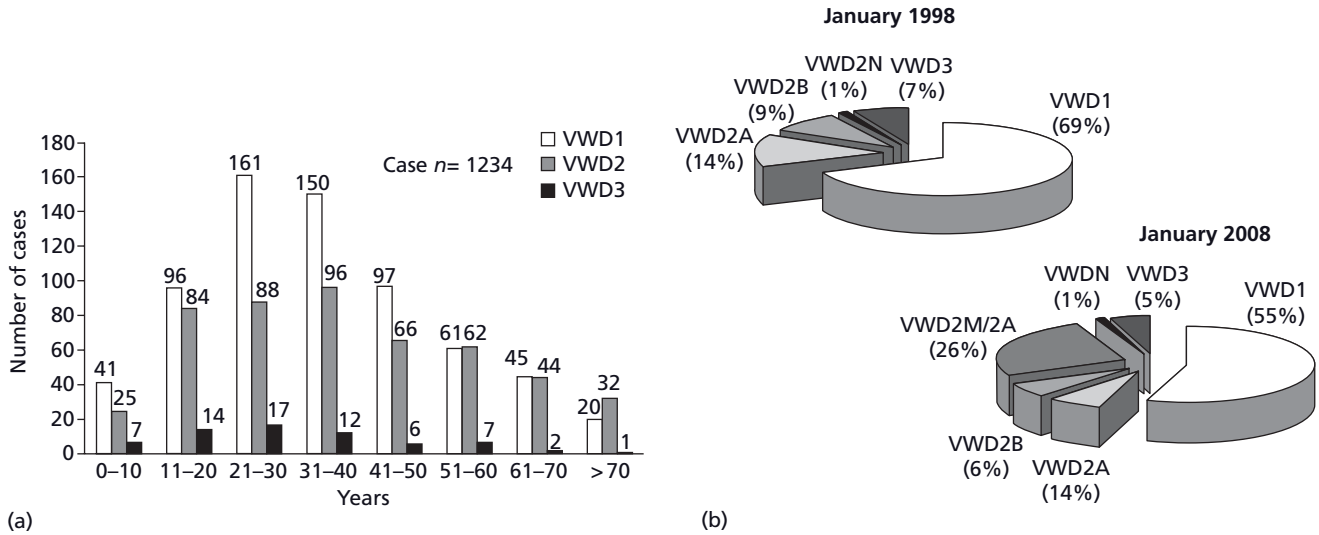

Figure 45.1 Age distribution of the 1234 patients enrolled in the Italian Registry on VWD. Each decade reports the frequency of VWD1, VWD2, and VWD3 (upper panel). VWD type distribution of the same population, as classified in 2008 according to the recommendations reported in reference 2. Note that the percentage of VWD1 is lower than that found 10 years before by the Italian Association of Hemophilia Centers (lower panel).

Table 45.1 Prevalence (%) of bleeding symptoms in patients with VWD from different cohorts and in normal individuals (adapted from Federici [7]; Silwer [10]; Lak *et al*. [11]).

	Iranian	Italian[a]			Scandinavia	
	Type 3 (n = 348)	Type 1 (n = 671)	Type 2 (n = 497)	Type 3 (n = 66)	VWD (n = 264)	Normal (n = 500)
Epistaxis	77	61	63	66	62	5
Menorrhagia	69	32	32	56	60	25
Postextraction bleeding	70	31	39	53	51	5
Hematomas	n. r.	13	14	33	49	12
Bleeding from minor wounds	n. r.	36	40	50	36	0.2
Gum bleeding	n. r.	31	35	56	35	7
Postsurgical bleeding	41	20	23	41	28	1
Postpartum bleeding	15	17	18	26	23	19
Gastrointestinal bleeding	20	5	8	20	14	1
Joint bleeding	37	3	4	45	8	0
Hematuria	1	2	5	12	7	1
CNS bleeding	n. r.	1	2	9	n. r.	0

n. r., not reported.

[a]Bleeding symptoms in Italian patients have been recently recalculated according to the updated results of the Italian Registry of VWD and therefore are different from those previously reported [2].

Several attempts were recently made to evaluate sensitivity and specificity of categorized bleeding symptoms to predict a VWD diagnosis, especially among mild cases thought to be VWD1 (VWF:RCo levels > 20 U/dL). In a multicenter study carried out in obligatory carriers of VWD1, menorrhagia and epistaxis were poor predictors of the disease whereas cutaneous bleeding and bleeding after dental extractions were more sensitive predictors of this diagnosis [12].

A bleeding severity score (BSS) has been analyzed in affected and nonaffected members of 154 families enrolled prospectively in a large European study on VWD1 [13]. The list of symptoms considered in the detailed questionnaire together with their severity degree calculated from –1 to 4 are summarized in Table 45.2. Despite the fact that this BSS was originally investigated in a large cohort of patients with VWD1, this approach can be useful in all types of VWD [14,15].

Table 45.2 Bleeding score used to the evaluate the bleeding history (see [13]).

	Score					
Symptom	–1	0	1	2	3	4
Epistaxis	–	No or trivial (less than 5)	>5 or more than 10′	Consultation only	Packing or cauterization or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Cutaneous	–	No or trivial (<1 cm)	>1 cm and no trauma	Consultation only		
Bleeding from minor wounds	–	No or trivial (less than 5)	>5 or more than 5′	Consultation only	Surgical hemostasis	Blood transfusion or replacement therapy or desmopressin
Oral cavity	–	No	Referred at least one	Consultation only	Surgical hemostasis or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Gastrointestinal bleeding	–	No	Associated with ulcer, portal hypertension, hemorrhoids, angiodysplasia	Spontaneous	Surgical hemostasis, blood transfusion, replacement therapy, desmopressin, antifibrinolytics	
Tooth extraction	No bleeding in at least two extractions	None done or no bleeding in one extraction	Referred in <25% of all procedures	Referred in >25% of all procedures, no intervention	Resuturing or packing	Blood transfusion or replacement therapy or desmopressin
Surgery	No bleeding in at least two surgeries	None done or no bleeding in one surgery	Referred in <25% of all surgeries	Referred in >25% of all procedures, no intervention	Surgical hemostasis or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Menorrhagia	–	No	Consultation only	Antifibrinolytics, pill use	Dilatation and currettage, iron therapy	Blood transfusion or replacement therapy or desmopressin or hysterectomy
Postpartum hemorrhage	No bleeding in at least two deliveries	No deliveries or no bleeding in 1 delivery	Consultation only	Dilatation and currettage, iron therapy, antifibrinolytics	Blood transfusion or replacement therapy or desmopressin	Hysterectomy
Muscle hematomas	–	Never	Post trauma no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion
Hemarthrosis	–	Never	Post trauma no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion
CNS bleeding	–	Never	–	–	Subdural, any intervention	Intracerebral, any intervention

The diagnosis of VWD may require several laboratory tests to be repeated on different occasions. These tests are usually performed on patients with suspected bleeding disorders. Table 45.3 provides an algorithm for VWD diagnosis. The bleeding time (BT), the original *in vivo* test used for diagnosing VWD, is not always prolonged and may be normal in patients with mild VWD, such as those with VWD1 and normal platelet VWF content [2]. Hence, it is not particularly useful for diagnosis. Evaluation of closure time (CT) with the platelet function analyzer (PFA-100) gives rapid and simple measure of VWF-dependent platelet function at high shear stress: it can be performed in whole blood and therefore can be employed instead of the BT in children or when the BT is not feasible. This system is sensitive and reproducible for VWD screening, but the CT is normal in VWD2N and cannot be modified in VWD3 after the administration of VWF/FVIII concentrates [16]. Based on these observations, BT and CT were not included in the flow chart to be used in the differential diagnosis of VWD types (Figure 45.2).

Molecular and prenatal diagnoses of VWD have been introduced since the early 1990s. Originally, the first mutations were found within exon 28 of VWF gene that is responsible for domains A1, A2, and A3. Most VWD2A cases are because of missense mutations in the A1 domain, with R1597W or Q or Y and S1506L accounting for about 60% of them [17]. The majority of VWD2B cases are because of missense mutations in the A1 domain, about 90% being caused by R1306W, R1308C, V1316M, and R1341Q [17]. A few heterogeneous mutations, also located within the A1 domain, cause VWD2M. A recurrent mutation in VWD1/2M Vicenza has been identified in families from Europe (R1205H) while a separate mutation (M740I) is seen exclusively in families from the Vicenza area in the north-east of Italy [18,19]. Missense mutations in the FVIII-binding domain at the amino-terminal portion of VWF are responsible for VWD2N [20]. The molecular defects responsible for VWD2 are located in specific VWF domains (Figure 45.3); on the other hand, mutations responsible for VWD1 and VWD3 are spread throughout the entire *VWF* gene.

The genetic causes of VWD1 are still elusive in many cases, especially in those with a mild phenotype. More information on the molecular basis of VWD1 has been collected by two multicenter international studies: in the European study, recruitment was based on the historical diagnosis of VWD1, which included 278 affected cases and 312 nonaffected family members [8]. The Canadian study describes 123 families in which an index case had bleeding symptoms with VWF levels between 5 and 50 U/dL [21]. In this latter study, subjects with abnormal multimeric patterns or other evidence of qualitative defects were excluded. The most important conclusions from both studies are the following: (i) despite the selection of

Table 45.3 Clinical and laboratory parameters used for VWD diagnosis.

(i) *Patients at risk for VWD*
Clinical history: lifelong mucocutaneous and postoperative bleeding, to be collected with appropriate questionnaires to calculate the Bleeding Severity Score (BSS)
Screening tests: prolonged bleeding time (maybe normal); normal platelet count; prolonged PTT (maybe normal)
(ii) *Diagnosis and definition of VWD type*
VWF antigen
VWF:Ristocetin cofactor activity
Factor VIII
VWF multimeric structure on low-resolution gels
(iii) *Characterization of VWD type*
Ristocetin-induced platelet agglutination (RIPA)
VWF multimeric structure on high-resolution gels
Platelet VWF content
Factor VIII binding assay

For the use of these tests see the diagnostic flow-chart reported in Figure 45.2 and also in [2] and [7].

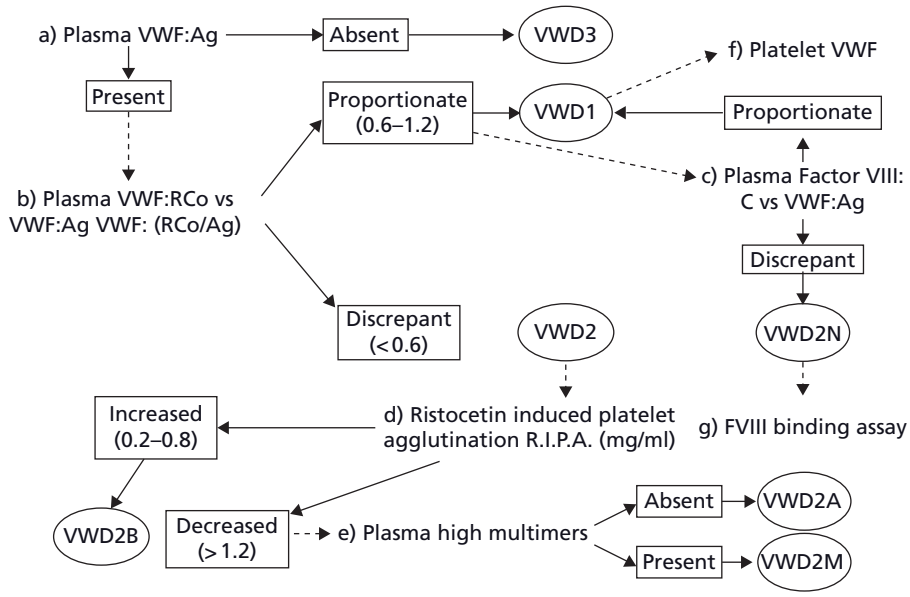

Figure 45.2 Algorithm proposed for the diagnosis of different VWD types. VWD3 can be diagnosed in case of undetectable VWF:Ag (a). A proportionate reduction of both VWF:Ag and VWF:RCo with a VWF:RCo/Ag ratio > 0.7 suggests VWD1 (b). If the VWF:RCo/Ag ratio is <0.7 type 2 is diagnosed. VWD2B (d) can be identified in case of heightened RIPA (<0.8 mg/ml) whereas VWD2A and VWD2M cause low RIPA (>1.2 mg/ml). Multimeric analysis in plasma (e) is necessary to distinguish between VWD2A (lack of the largest and intermediate multimers) and VWD2M (all the multimers present). VWD2N can be suspected in case of discrepant values for FVIII (c) and VWF:Ag (ratio < 0.5) and diagnosis should be confirmed by the specific test (g) of VWF:factor VIII binding capacity (VWF:FVIIIB). In VWD1 the ratio between factor VIII and VWF:Ag is always ≥1 and the severity of VWD1 phenotype can usually be evaluated from platelet VWF (f) measurements. This figure is derived from that originally reported previously [2].

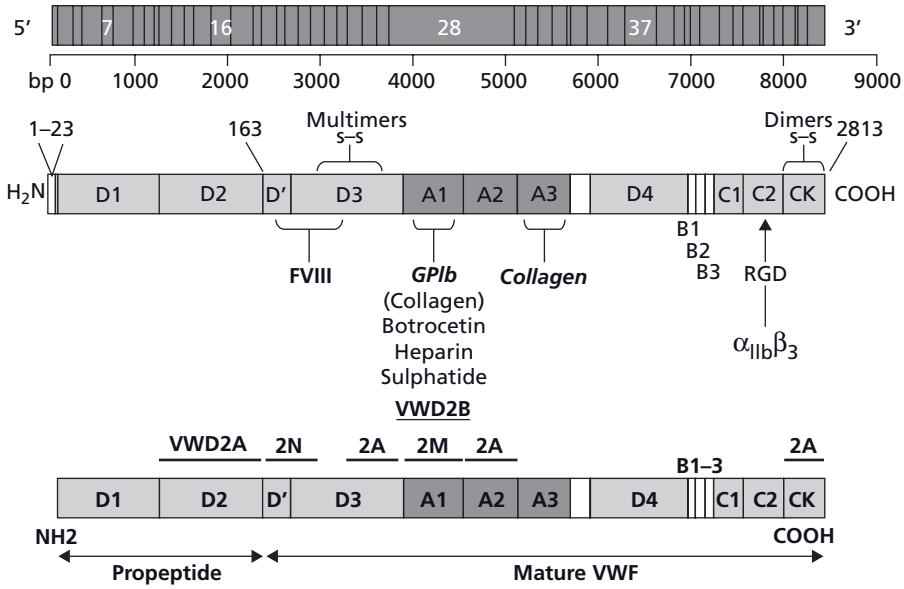

Figure 45.3 Schematic representation of the *VWF* gene located in chromosome 12: the main exons are indicated with the number of base pairs from 5′ to 3′ (upper panel). The structure of VWF functional domains: the pre-pro-VWF is indicated with amino acids numbered from the amino- (aa 1) to carboxy-terminal portions (aa 2813) of VWF. Note the important CK and D3 domains for formation of VWF dimers and multimers. The native mature subunit of VWF, after the cleaving of the pre-pro VWF, is described with its functional domains: the VWF binding sites for factor VIII (D′ and D3), GpIb, botrocetin, heparin, sulfatide, collagen (A1), collagen (A3), and the RGD sequence for binding to αIIbβ3 (intermediate panel). Distribution of VWF mutations in patients with VWD types 2: the positions of mutations causing VWD2A, VWD2B, VWD2M, and VWD2N are indicated with black bars throughout the VWF domains (lower panel).

patients based on bleeding history, candidate VWF mutations were not found for 27% (Canadian) and 36% (European) of index cases diagnosed with VWD1; and (ii) the spectrum of VWD1 mutations was different from that found in VWD3. Therefore, VWD1 is not at all like heterozygous VWD3 because VWF defects that occur in VWD1 usually are caused by dominant VWF abnormalities that affect VWF secretion or clearance without altering multimeric patterns or platelet binding.

In VWD3, partial or total gene deletions have been initially reported [22]. Notably, homozygosity for gene deletion may be associated with the appearance of allo-antibodies against VWF, which may render replacement therapy ineffective and stimulate anaphylactic reactions to treatment [23,24]. In general, mutations may be scattered over the entire gene, but some (e.g., 2680delC or Arg2535X) are particularly prevalent, especially in northern Europe [25]. Gene defects of VWD3 patients from three different populations have now been studied; however, no founder effect was observed and mutations were distributed throughout the entire *VWF* gene [26].

Compared with hemophilia, most VWD patients show relatively mild bleeding symptoms. Therefore, prenatal diagnosis is recommended when parents are known to be carriers of VWD3 based on gene defects identified in their first affected child. Since young children with VWD3 might carry deletions of *VWF* gene that predispose to the allo-antibodies to VWF, every new child with VWD3 should be genetically investigated for deletions before starting extensive therapy with exogenous VWF concentrates.

Clinical definition of severe versus mild von Willebrand disease

In both retrospective and prospective studies VWD3 is defined as severe when undetectable VWF levels are measured in both plasma and platelets and when low amounts of FVIII:C (<20 U/dL) are present. Conversely, VWD1 and VWD2 are very heterogeneous and their clinical presentation is strictly correlated with the circulating levels of a functional VWF, measured as ristocetin cofactor activity (VWF:RCo).

In the last 5 years the results of an international prospective study on the use of desmopressin (DDAVP) in "severe forms of VWD" has been reported [27]. For the first time the steering committee of that study made an attempt to define patients with "severe VWD," using levels of VWF and FVIII activities as an index of severity, following the same criteria used for hemophiliacs. "Severe VWD" was ascribed to those patients who had a lifelong history of bleeding (including at least two episodes of blood loss severe enough to require replacement therapy) and at least one of the following laboratory abnormalities: VWF:RCo < 10 IU/dL and/or FVIII:C < 20 IU/dL. Therefore, the "severe VWD" group included not only VWD3 but also VWD1, VWD2A, VWD2M, and VWD2N with low VWFRCo and/or FVIII:C levels [27].

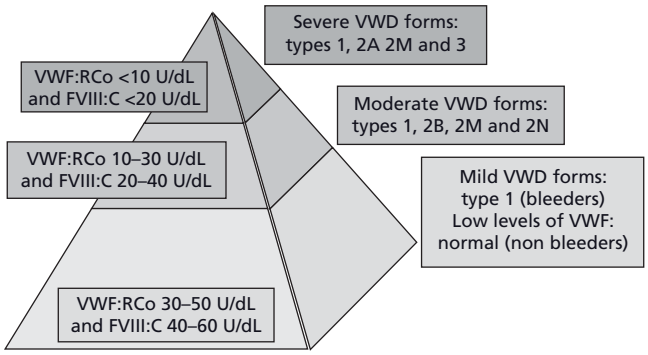

Figure 45.4 Pictorial representation of the three different degrees of VWD severity according to levels of VWF and FVIII activities: severe, moderate, mild VWD. In the upper part of the pyramid, the most "severe VWD" (VWD3, VWD2A, VWD1) are included with levels of VWF:RCo < 10 U/dL and/or FVIII:C < 20 U/dL. The "moderate VWD" (VWD2B, VWD2M, VWD2N, and VWD1) with levels of VWF:RCo 10–30 U/dL and/or FVIII:C 20–40 U/dL and the "mild VWF" (VWF:RCo 30–50 U/dL and/or FVIII:C 40–60 U/dL) are described in the middle portion and in the base of the pyramid. Owing to the physiologic changes of VWF and of the variability of the assays, a very "mild VWD" with values of VWF:RCo and/or FVIII:C > 40 U/dL can be diagnosed only when these low-borderline levels of VWF and FVIII activities are associated with personal and family bleeding history. Therefore, the lower the levels of VWF:RCo or FVIII:C the higher is the probability of a correct VWD diagnosis: in this sense, the "severe VWD" might represent the "tip of the iceberg" of a large number of moderate and mild VWF defects.

Similar criteria have been used by the steering committee of the Italian National Registry on VWD to evaluate retrospective data on 1234 VWD patients collected from 16 hemophilia centers on behalf of the Italian Association of Hemophilia Centers [15].

Utilizing such a definition of "VWD severity" that is based on the levels of defective VWF:RCo and/or FVIII:C, three different groups of VWD can be identified: a first group of "*severe VWD*" with VWF:RCo < 10 U/dL and/or FVIII:C < 20 U/dL [7]; a second group of "*moderate VWD*" with VWF:RCo 10–30 U/dL and/or FVIII:C 20–40 U/dL; a third group of "*mild VWD*" with VWF:RCo 30–50 U/dL and/or FVIII:C 40–60 U/dL. A pictorial pyramidal representation of the three different degrees of VWD severity according to levels of both VWF:RCo and FVIII:C activities is shown in Figure 45.4.

The "severe VWD" might represent the "tip of the iceberg" overlying a large number of undiagnosed patients with mild VWF defects. While no diagnostic problems occur in moderate to mild VWD with levels of VWF:RCo < 40 U/dL, a definite diagnosis of VWD is often difficult to make in patients with very mild VWD forms and VWF:RCo levels > 40 U/dL. Moreover, it is well known that the physiologic changes of VWF levels and the variability of the VWF:RCo assays can obscure mild defects of VWF. In very mild VWD, the limit between "disease" and "reduced levels of VWF in a normal individual" can be difficult in the absence of bleeding history in other members of the family, as discussed previously [28].

By contrast, many mild VWF defects remain undiagnosed in the absence of well-documented personal and family bleeding histories since only the patients characterized by low VWF:RCo levels are easily recognized and therefore followed by the hemophilia centers (tip of the iceberg, in Figure 45.4). An analytic approach using Bayes theorem recently confirmed the need for three major criteria (bleeding, low VWF, and affected family members) in the diagnosis of VWD1 [9]. More recently, the use of BSS and threshold levels of VWF:RCo and FVIII have been investigated in a prospective study organized in a large cohort of Italian patients with different VWD types [15]. In this study of 814 VWD cases, BSS > 10, VWF:RCo < 10 U/dL, and FVIII < 20 U/dL were significantly associated with high risk of bleeding. By multivariate modeling which included all variables, BSS > 10 was the most significant predictor of bleeding. On the other hand, VWD patients with VWF:RCo > 30 U/dL and FVIII > 40 U/dL and 5 < BSS < 10 have the lowest incidence of bleeding [15].

Conclusions and future perspectives

von Willebrand disease is the most common inherited bleeding disorder, largely because of large heterogeneity of VWF defects. The clinical diagnosis can be difficult for the general hematologist to make because of the widely variable phenotype. Combining a clinical assessment of bleeding with an appropriate laboratory investigation can improve the capacity to diagnose the disease accurately. Molecular diagnosis can be useful to confirm specific VWF defects in VWD families. It is still not clear whether most mild VWD1 patients really have a mutation in the VWF locus. For this and other important residual scientific questions, large prospective studies are needed.

Acknowledgments

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References

1 Federici AB, Mannucci PM. Management of inherited von Willebrand disease in 2007. *Ann Med* 2007; **39**: 346–58.
2 Federici AB, Castaman G, Mannucci PM. Guidelines for the diagnosis and management of VWD in Italy. *Haemophilia* 2002; **8**: 607–21.

3 Pasi KJ, Collins PW, Keeling DM, *et al.* The diagnosis of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organization. *Haemophilia* 2004; **10**: 218–31.

4 Nichols WL, Hulting MB, James AH, *et al.* Von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the national Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA). *Haemophilia* 2008; **14**: 171–232.

5 Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. *Blood* 1987; **69**: 454–9.

6 Sadler JE, Budde U, Eikenboom JCJ, *et al.* Update on the pathophysiology and classification of von Willebrand disease. A report of the Subcommittee on von Willebrand factor. *J Thromb Haemost* 2006; **4**: 2103–14.

7 Federici AB. Clinical diagnosis of von Willebrand disease. *Haemophilia* 2004; **10**(Suppl. 4): 169–76.

8 Goodeve A, Eikenboom J, Castaman G, *et al.* Phenotype and genotype of a cohort of families historically diagnosed with type 1 von Willebrand disease in the European study, Molecular and Clinical Markers for the Diagnosis and Management of Type 1 von Willebrand disease. *Blood* 2007; **109**: 112–21.

9 Tosetto A, Castaman G, Rodeghiero F. Evidence-based diagnosis of type 1 von Willebrand disease: a Bayes theorem approach. *Blood* 2008; **111**: 3998–4003.

10 Silwer J. von Willebrand's disease in Sweden. *Acta Paediat Scand* 1973; **238**: 1–159.

11 Lak M, Peyvandi F, Mannucci PM. Clinical manifestations and complications of childbirth and replacement therapy in 348 Iranian patients with type 3 von Willebrand disease. *Br J Haematol* 2000; **111**: 1223–9.

12 Rodeghiero F, Castaman G, Tosetto A, *et al.* The discriminant power of bleeding history for the diagnosis of von Willebrand disease type 1: an international multicenter study. *J Thromb Hemost* 2005; **3**: 2619–26.

13 Tosetto A, Rodeghiero F, Castaman G, *et al.* A quantitative analysis of bleeding symptoms in type 1 of von Willebrand disease: results from a multicenter European Study (MCMDM-1VWD). *J Thromb Hemost* 2006; **4**: 774–82.

14 Federici AB, Mannucci PM, Castaman G, *et al.* Clinical and molecular predictors of thrombocytopenia and risk of bleeding in patients with von Willebrand disease type 2B: a cohort study of 67 patients. *Blood* 2009; **113**: 526–34.

15 Federici AB, Bucciarelli P, Castaman G, *et al.* Prevalence and determinants of bleeding in different types of von Willebrand disease: results of the first prospective multicenter study on 814 Italian patients. *Blood* 2007; Abstract 713.

16 Cattaneo M, Federici AB, Lecchi A, *et al.* Evaluation of the PFA-100 system in the diagnosis and therapeutic monitoring of patients with von Willebrand disease. *Thromb Haemost* 1999; **82**: 35–39.

17 Meyer D, Fressinaud E, Hilbert L, *et al.* Type 2 von Willebrand disease causing defective von Willebrand factor-dependent platelet function. *Best Pract Res Clin Haematol* 2001; **14**: 349–64.

18 Schneppenheim R, Federici AB, Budde U, *et al.* von Willebrand disease type 2 M "Vicenza" in Italian and German patients: identification of the first candidate mutation (G3864A; R1205H) in 8 families. *Thromb Haemost* 2000; **83**: 136–40.

19 Castaman G, Missiaglia E, Federici AB, *et al.* An additional candidate mutation (G2470A; M740I) in the original families with von Willebrand disease type 2 M Vicenza and the G3864A (R1205H) mutation. *Thromb Haemost* 2000; **84**: 350–1.

20 Mazurier C, Goudemand J, Hilbert L, *et al.* Type 2N von Willebrand disease: clinical manifestations, pathophysiology, laboratory diagnosis and molecular biology. *Best Pract Res Clin Haematol* 2001; **14**: 337–47.

21 James PD, Notley C, Hegadorn C, *et al.* The mutational spectrum of type 1 von Willebrand disease: results from a Canadian cohort study. *Blood* 2007; **109**: 145–54.

22 Shelton-Inloes BB, Chebab FF, Mannucci PM, Federici AB, Sadler JE. Gene deletion correlates with the 21 development of alloantibodies in von Willebrand disease. *J Clin Invest* 1987; **79**: 1459–65.

23 Mannucci PM, Tamaro G, Narchi G, *et al.* Life-threatening reaction to factor VIII concentrate in a patient with severe von Willebrand disease and alloantibodies to von Willebrand Factor. *Eur J Haemat* 1987; **39**: 467–70.

24 Mannucci PM, Federici AB. Antibodies to von Willebrand factor in von Willebrand disease. *Adv Exp Med Biol* 1995; **386**: 87–92.

25 Eikenboom JCJ. Congenital von Willebrand disease type 3: clinical manifestations, pathophysiology and molecular biology. *Best Pract Res Clin Haematol* 2001; **14**: 365–79.

26 Baronciani L, Cozzi G, Canciani MT, *et al.* Molecular characterization of a multiethnic group of 21 patients with type 3 von Willebrand disease. *Thromb Haemost* 2000; **84**: 536–40.

27 Federici AB, Mazurier C, Berntorp E, *et al.* Biological response to desmopressin in patients with severe type 1 and type 2 von Willebrand disease: results of a multicenter European study. *Blood* 2004; **103**: 2032–8.

28 Sadler JE. Von Willebrand disease type 1: a diagnosis in search of a disease. *Blood* 2003; **101**: 2089–93.

46 Women and von Willebrand disease

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Introduction

Studies have clearly documented a definite degree of obstetric [1–4] and gynecologic morbidity [5–8] in females with von Willebrand disease (VWD), particularly in the past decade (Table 46.1). The majority (≥80%) of these patients over the course of their menstrual life will develop menorrhagia [5–7]. Furthermore, up to a fifth of these patients have undergone surgical intervention such as hysterectomy for control of menorrhagia refractory to medical management [5,7]. In addition, the constancy of the menorrhagia clearly impairs quality of life [5,7]. This chapter will highlight and review relevant gender-specific epidemiologic and clinical characteristics and therapeutic issues in the patient with menorrhagia and/or postpartum hemorrhage related to VWD.

Epidemiology of von Willebrand disease in women

Understandably, case finding of VWD in women has focused on women presenting with menorrhagia. A systematic review by Shankar *et al.* [9] has summarized the overall prevalence of the laboratory diagnosis of VWD in women presenting with menorrhagia to be 13% (confidence intervals 11–15.6%) of a total of 988 women in 11 studies (Figure 46.1). However, this does not represent a worldwide prevalence of VWD since these studies have been primarily from Europe and North America. Recently, though, several well-conducted prevalence studies in India [10] and Taiwan [11] have reported a similar prevalence.

Regarding the younger patient with menorrhagia, there have been few prevalence studies for an underlying bleeding disorder in the adolescent menorrhagia population [12,13] and most of these studies have focused on the inpatient and emergency setting. One study included 106 adolescents in an inpatient and outpatient gynecology tertiary care setting [13]. The mean prevalence of VWD was 8% from a total of 332 adolescents. In a more recent study, in 61 adolescents referred for hemostasis evaluation to a Hemophilia Treatment Center, a prevalence of VWD of 36% (22/61) [95% confidence interval (CI) 24–49%] was found [14].

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Diagnostic aspects of von Willebrand disease in women

The results of the epidemiologic studies reviewed above have led to relatively widespread VWD testing in the female population [15]. However, the clinician must be aware that VWF and factor VIII (FVIII) levels can fluctuate [16]. Hormonal factors, both exogenous and endogenous, are a potential mechanism in part for such fluctuation [16]. Consequently, there are several subtleties in the laboratory diagnosis of VWD that warrant clarification.

Testing in relation to the menstrual cycle

Historically, there have been reports, albeit of a relatively small total number of patients [17], that overall pointed to a decrease in the VWF levels during menstruation. This has since been supported by two more recent studies [18,19], and although another study by cross-sectional analysis showed no decrease in the levels during menses [20], it is possible that the sampling was not early enough in the menstrual cycle. Consistent with the predominant findings from multiple studies, Miller *et al.* [19] reported a statistically significant decrease in VWF levels by cross-sectional analysis during the first four days of menses.

Testing in relation to combination oral contraceptive use

Historically, it has been felt that oral contraceptive (OC) use can obscure the diagnosis of VWD based on an observation that estrogen can raise the VWF levels in VWD patients [21]. However, presently there is lack of evidence demonstrating a definite effect on VWF levels of the current combination OCs (which are of lower dose potency than the estrogen preparations associated with the initial case reports of estrogen raising the VWF levels). One recent study, by contrast, described a dampening of the cyclic fluctuation of the VWF levels among women on low-dose OCs [18].

Table 46.1 von Willebrand disease-related complications in females.

Menstrual related	Other menstrual issues	Childbirth related
Majority with menorrhagia	Increased prevalence of mid-cycle pain (Mittlesmerz) and dysmenorrhea	Postpartum hemorrhage <24 h
Iron-deficiency anemia	Risk of hemoperitoneum	Postpartum hemorrhage >48 h up to 4 weeks
Increased rate of surgical interventions: D&C, hysterectomy	?Increased incidence of endometriosis, polyps, fibroids	Vulvar hematoma
Decreased quality of life (increased time lost from school/work, ?increased anxiety/depression)		

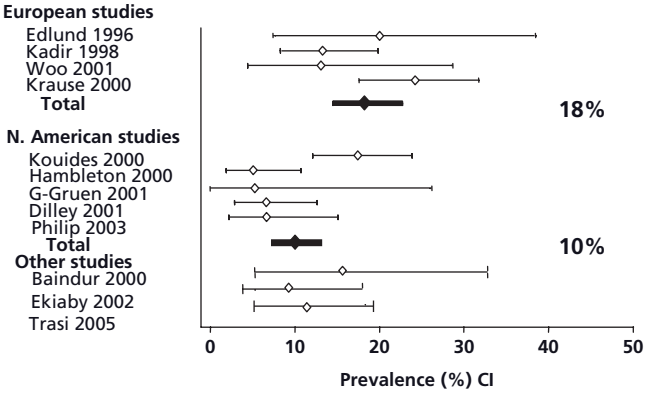

Figure 46.1 Prevalence of von Willebrand disease in adult females presenting with menorrhagia. Adapted from Shankar *et al.* [9].

Other laboratory issues

Other factors that may have an impact on the laboratory diagnosis of VWD in women are race and age. Recent studies have demonstrated significantly higher VWF levels in black women [18,22] even after adjusting for the blood group. There is also an age-dependent increase in the VWF level independent of race [18]. However, the mean age of this study group was 26 ± 5.5 years so it is not known if this increase of VWF levels continues toward menopause.

Clinical characteristics of von Willebrand disease in women

Women with VWD certainly have a very high relative risk of menorrhagia compared with the general population, and the prevalence of menorrhagia by subjective report has been between 78% and 97% in women primarily with type 1 VWD [5–7]. Using the more objective pictorial blood assessment chart (PBAC) [23], it has been reported that 78% of patients with VWD (primarily type 1) had evidence for menorrhagia [5].

Table 46.2 Significant bleeding symptoms in women with von Willebrand disease.

	Study population	Symptoms more common in VWD patients than in non-VWD patients
Royal Free London, 1998 [25]	26/150 women presenting with menorrhagia subsequently diagnosed with VWD (n = 26, presumed all type 1)	Bruising Dental-related bleeding Surgical-related bleeding Postpartum hemorrhage Menorrhagia since menarche Multiple bleeding symptoms
Upstate NY Hemophilia Treatment Centers study, 2000 [7]	81 menstruating women registered at hemophilia treatment centers, all type 1 VWD compared with a cohort of 150 menstruating volunteers	Age (the younger the age, the higher the probability of VWD) History of dental-work-related bleeding Past or present history of anemia A diminished quality of life during menses in relation to family activities
Centers for Disease Control—Atlanta, 2002 [8]	102 women with VWD registered at hemophilia treatment centers compared with 88 controls	Surgical-related bleeding Excessive gum bleeding Bleeding after minor injuries

Women with VWD use more tampons and pads than non-VWD menstruating women and have frequent staining of underclothes [7] and a higher prevalence of anemia (28–66%) [6,7,24]. These women also have a much higher frequency of other mucocutaneous bleeding symptoms. Table 46.2 summarizes the prevalence of these bleeding symptoms in comparison with a control group of non-VWD women from several recent studies [7,8,25].

Women with type 2 and type 3 VWD have not been as extensively studied as the type 1 patients. However, a study on behalf of the International Society of Thrombosis and Hemostasis (ISTH) von Willebrand factor subcommittee reported a high prevalence of menorrhagia and a quarter of the patients necessitated hysterectomy [26]. Subsequent studies have focused on the more common type 1 patient and even in this population with the milder depression of the VWF level there is a relatively high rate (8–26%) of hysterectomy for control of menorrhagia among type 1 patients [5–8,24]. In a case–control study of 102 women with VWD carried out by the Centers for Disease Control (CDC), 26% had undergone hysterectomy compared with 9% of controls [8]. In two studies, there was also underlying uterine pathology noted in the hysterectomy specimen, where it can be postulated that mild VWD may "unmask" a uterine fibroid [5,7]. In the CDC

study, a statistically higher rate of fibroids compared with age-matched controls (32% vs. 17%) was noted [8]. There may also be a higher prevalence of endometriosis (30% vs. 10%), endometrial hyperplasia (10% vs. 1%), and endometrial polyps (8% vs. 1%) in VWD women compared with controls [8]. It has been hypothesized that VWD may exacerbate the presumed retrograde menstrual flow implicated in the pathophysiology of endometriosis [27]. The higher prevalence of endometrial hyperplasia and polyps in VWD women has been explained in terms of the VWD "unmasking" these lesions [27].

Regarding psychosocial aspects, several studies comprising over 300 patients with VWD compared with non-VWD women has shown unequivocally that these women do have impaired quality of life [7,8,24,28,29]. Dysmenorrhea has been noted in approximately half [7,24]. A high rate of mid-cycle pain, termed "mittlesmerz," has also been noted in women with VWD [7] and these patients can develop an acute surgical abdomen from hemiperitoneum because of bleeding into the corpus luteum with subsequent rupture [30]. A report from Sweden showed that 9 of 136 women with VWD (6.8%) experienced hemorrhagic ovarian cysts [31]. There have also been reports of bleeding into the broad ligament with the patient presenting with a positive ilio-psoas sign [1].

Management of von Willebrand disease-related menorrhagia

An algorithm for the management of menorrhagia adapted from a recent consensus panel is presented in Figure 46.2 [32].

Menorrhagia management: hemostatic agents (antifibrinolytic therapy, desmopressin, von Willebrand factor-containing plasma concentrates)

Despite the widespread use of tranexamic acid (TA) for decades for the general menorrhagia population [33], there remains a paucity of adequate objective data on the efficacy of this treatment to reduce menstrual blood loss (MBL) in women with VWD [34–36]. At the Royal Free Hospital in London, TA was successful as a first-line therapy in only 40% of 37 women with bleeding disorder-related menorrhagia by reducing MBL to a PBAC score of <100 or to a woman's satisfaction [37]. There is also a new sustained-release formulation (XP12B) of TA that could be dosed only two to three times a day. This is currently under study in the USA [38].

In nonrandomized cohort studies, desmopressin (DDAVP) has been reported by patient self-assessment as "excellent"/"very effective" in approximately two-thirds of patients with either the subcutaneous [39] or intranasal (IN) use [40]. However, in 30 women with VWD-related menorrhagia using the PBAC in a randomized control crossover study, there was no difference in bleeding severity when IN-DDAVP was compared with placebo [41]. Regardless of whether the first treatment period involved the placebo or the IN-DDAVP, there was a reduction in the PBAC score that was statistically significant ($P = 0.01$). Similar results were noted in a related study of 20 women with menorrhagia and a prolonged bleeding time comparing 300 μg of IN-DDAVP with placebo [42]. There was no statistically significant decrease in menstrual blood flow spectrophotometrically with DDAVP compared with the placebo. However, there was a statistically significant decrease in bleeding when DDAVP was combined with TA [42].

In summary, more objective measurements of efficacy have not shown as great a benefit of IN-DDAVP for VWD-related menorrhagia compared with prior studies using subjective assessment as the endpoint of efficacy. However, a recent multicenter US crossover trial of women with abnormal laboratory hemostasis (including VWD) comparing IN-DDAVP and TA using the PBAC for assessment of MBL and four previously validated quality of life (QoL) measures has been completed [43]. Both medications reduced menstrual flow and improved QoL among females with menorrhagia and abnormal laboratory hemostasis, but TA proved to be more effective than IN-DDAVP [43]. At least one important question remains regarding the efficacy of DDAVP: Can outcomes be improved without an increase in the adverse event rate by altering the dosing schedule? For example, does changing standard dosing from one puff in each nostril on days 2 and 3 of menses to a

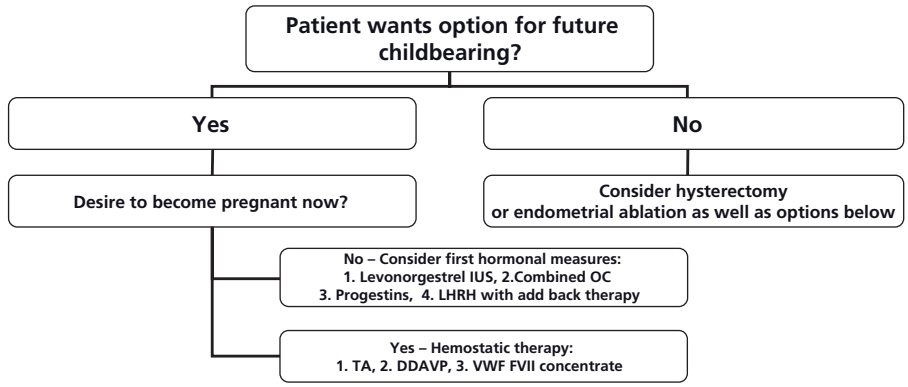

Figure 46.2 Algorithm of von Willebrand disease-related menorrhagia. Adapted from James *et al.* [32].

dosing schedule of twice a day for the first two days of menses, daily for three days, or one puff to each nostril on days 2–7 improve control of bleeding? (Edlund, M. Gyn Karolinska Hospital, Stockholm, Sweden, personal communication). In addition, further study of the efficacy and safety of combined therapy of IN-DDAVP and antifibrinolytic therapy or hormonal therapy is also needed.

Approximately 10–15% of women with VWD will not respond to DDAVP because they either have severe type 1 VWD or type 2 or type 3 VWD [44]. In those patients, for severe intractable menorrhagia refractory to antifibrinolytic therapy and/or hormonal therapy or for prophylaxis before surgery, a plasma-derived VWF-containing FVIII concentrate can be administered [44]. The dosing is typically is 40–60 U/kg of VWF:RCo units for major surgery and 20–40 U/kg of VWF:RCo units for menorrhagia.

Preliminary data from the Swedish type 3 VWD prophylaxis registry show that there are patients requiring prophylaxis for the indication of control of menorrhagia [45]. In order to accrue further data and test a strategy of escalated prophylaxis, an international prophylaxis study [46] was initiated last year in women with type 2 and 3 VWD-related menorrhagia with dosing initially at 50 U/kg of VWF:RCo units weekly. In nonresponders (PBAC remaining >100) the dose is escalated to twice a week then to three times per week.

Menorrhagia management: hormonal therapy

Combined OC is useful for cycle regulation and inhibition of the growth and development of the endometrium. On the other hand, the efficacy of OC in reducing the menstrual loss in women with VWD or other bleeding disorders has not been well established. In a survey of type 2 and 3 VWD patients by Foster *et al.* [26], 88% of women treated with OCs stated that it was effective. However, in type 1 VWD patients a standard dose of OCs was effective only 24% of the time while high-dose OC therapy was effective only 37% of the time [7]. On other hand, combined OCs have the added advantages of excellent contraception, good cycle control, and reduction in the incidence of dysmenorrhea, premenstrual tension, and cyst rupture. The latter (hemoperitoneum) is a rare but potentially life-threatening event in women with VWD [30]. In light of those additional benefits, the 2008 National Heart Lung Blood Institute VWD treatment guidelines [47] advise front-line use of OC therapy for the adult or adolescent who does not desire pregnancy but may desire future childbearing.

Besides OCs, progestins [37] may also be useful in high doses alone or in combination with DDAVP or factor concentrate to arrest acute menorrhagia. A new emerging option is the levonorgestrel intrauterine system, Mirena (LNG-IUS). In a recent study at the Royal Free hospital, LNG-IUS was assessed in 16 women (13 with type 1 VWD, two with FXI deficiency, and one with platelet storage disorder) with bleeding disorders [48]. At 9 months of follow-up, nine women became amenorrheic and in the remaining seven the PBAC score ranged from 24 to 75 (median 47; $P = 0.0001$). Long-term follow-up at a median of 53 months has shown persistent benefit [49]. Whether the underlying bleeding disorder promotes prolonged spotting (median 42 days, 30–90 days [48]), as recently reported in one case [50], deserves further study as persistent spotting is an indication for removal.

Obstetric aspects of von Willebrand disease

It has been well established that part of the "physiologic response" in pregnancy is a progressive elevation of the factor VIII and VWF levels [51]. However, it should be stressed that there is proportionately less elevation in the levels compared with those in the normal pregnant patient. Consequently, and not surprisingly, a higher rate of postpartum hemorrhage (PPH) (16–29% vs. 3–5% in the general population [2]) has been reported in the first 24 h following delivery. This meets or exceeds the criteria for primary PPH [1,2,4]. Also, there appears to be a higher rate in type 2 and 3 patients than in type 1 patients [1,2,4]. This may result in the need for red cell transfusions in 7–17% [4,7] of type 1 patients.

In a recent analysis of the United States Nationwide Inpatient Sample involving 4067 deliveries among women with VWD (1 in 4000 deliveries), James and Jamison observed that women with VWD were more likely to experience a PPH [odds ratio (OR) 1.5; 95% CI 1.1–2.0], and had a fivefold increased risk of being transfused (OR 4.7; 95% CI 3.2–7.0). They also observed that women with VWD were more likely to experience antepartum bleeding (OR 10.2; 95% CI 7.1–14.6). Five of the 4067 women with VWD died, a maternal mortality rate 10 times higher than that for other women [52]. Besides an increased rate of PPH in VWD women, perineal hematomas appear to be more common [1,4].

The rise in the factor VIII and VWF levels occur in the second and third trimester so the clinician should not assume that there will not be excessive bleeding during amniocentesis or a first trimester abortion as levels may not have increased at this early gestational time point [53]. The presentation of VWD as transfusion-dependent bleeding at miscarriage has been reported [4]. Excessive bleeding beyond a week post partum up to 5 weeks post partum has also been reported [3,7,54,55] with an average onset of delayed hemorrhage in one study of 15.7 + 5.2 days [3]. Consequently, it should be common practice to remind a postpartum patient after childbirth of this possibility, which may occur as late as 4–6 weeks post delivery.

Management of von Willebrand disease during pregnancy

As noted above, gestational palliation does occur during pregnancy so that if such a patient needs a dental extraction or

other invasive procedure during pregnancy, DDAVP is not necessary if the VWF levels have "normalized." Even for women whose levels have not exceeded 50% there are, however, theoretic concerns for administering DDAVP pre partum for the following reasons:

- vasoconstrictive effect leading to decreased placental flow;
- risk of premature labor as DDAVP has weak V2 receptor activity; and
- risk of neonatal hyponatremia.

There has been a report documenting several cases of neonatal hyponatremia [56], but this is uncommon. There seems to be a sense among practitioners that DDAVP use pre partum is safe and that a dose during labor does not first require clamping of the umbilical cord. Mannucci has noted no adverse events associated with DDAVP use given in the second trimester in 31 women with VWD who underwent chorionic villus sampling or amniocentesis [57]. Sanchez-Lucero *et al.* noted no adverse events in 37 VWD women receiving DDAVP post partum [58]. Despite the expected aggressive fluid resuscitation women receive during the peripartum periods, there were no reports of symptomatic hyponatremia. Besides DDAVP, antifibrinolytic therapy is an option for control of PPH [59]. In addition, effective use of uterine artery embolization to treat a life-threatening hemorrhage in a type 3 VWD patient has been reported [60].

Regarding epidural analgesia, three single institution studies (total $n = 35$) in the past 3 years have shown that epidural anesthesia is safe if the VWF levels have normalized by the third trimester either physiologically or by medical intervention at the time of delivery [61–63].

In patients with type 2 or type 3 VWD, there is evidence that observation alone without treatment at the time of delivery can be considered provided that the factor VIII level is above 50%. This is based on the premise that the factor VIII level predicts deep tissue hemostasis as opposed to the VWF level. However, this is based only on a cohort of six women [54]. As such, most clinicians would be more comfortable to infuse a plasma-derived VWF-containing concentrate [55]. Theoretically, there could be concern for postpartum thrombosis, though reports of thrombosis with VWF concentrates have only been in the orthopedic setting. Nonetheless, pregnancy can be considered to a prothrombotic state of similar magnitude. There has been one report of thrombosis with DDAVP use in a type 2B patient during the postpartum period [64]. Dosing at delivery has typically been 40–80 U/kg. Postpartum prophylaxis has usually targeted a dose of 20–40 U/kg of a plasma-derived VWF-containing concentrate for at least 1 week post partum [55].

In the case of VWD Normandy and type 2B VWD, management has been problematic. The use of recombinant factor VIII concentrate has been reported in a type 2N patient with a factor VIII level < 50% at the time of delivery [65]. In the case of type 2B, platelet transfusions were given at the time of delivery when the platelet count fell as low as 20 000/mL [66].

Regarding the risk of miscarriage, the rate has been shown to be approximately ~20% in both type 1 and type 2 and 3 patients [4,27]. This rate may be slightly higher than the general population rate of 12–14% [27]: Direct age-matched comparisons with the general population have not been made except in one study from the CDC in 86 VWD women and 70 controls where a higher miscarriage rate of 15% was noted compared with 9% ($P = 0.005$) [8].

References

1 Greer IA, Lowe GD, Walker JJ, Forbes CD. Haemorrhagic problems in obstetrics and gynaecology in patients with congenital coagulopathies. *Br J Obstet Gynaecol* 1991; **98**: 909–18.

2 Ramsahoye BH, Davies SV, Dasani H, Pearson JF. Obstetric management in von Willebrand's disease: a report of 24 pregnancies and a review of the literature. *Haemophilia* 1995; **1**: 140–4.

3 Roque H, Funai E, Lockwood CJ. von Willebrand disease and pregnancy [Review]. *J Matern Fetal J*. 2000; **9**: 257–66.

4 Kadir RA, Lee CA, Sabin CA, Pollard D, Economides DL. Pregnancy in women with von Willebrand's disease or Factor XI deficiency. *Br J Obstet Gynaecol* 1998; **105**: 314–21.

5 Kadir RA, Economides DL, Sabin CA, Pollard D, Lee CA. Assessment of menstrual blood loss and gynaecological problems in patients with inherited bleeding disorders. *Haemophilia* 1999; **5**: 40–8.

6 Ragni MV, Bontempo FA, Cortese Hassett A. von Willebrand disease and bleeding in women. *Haemophilia* 1999; **5**: 313–17.

7 Kouides PA, Burkhart P, Phatak P, *et al.* Gynecological and obstetrical morbidity in women with type 1 von Willebrand disease: results of a patient survey. *Haemophilia* 2000; **6**: 643–8.

8 Kirtava A, Drews C, Lally C, Dilley A, Evatt B. Medical, reproductive and psychosocial experiences of women diagnosed with von Willebrand's disease receiving care in haemophilia treatment centres: a case–control study. *Haemophilia* 2003; **9**: 292–7.

9 Shankar M, Lee CA, Sabin CA, Economides DL, Kadir RA. von Willebrand disease in women with menorrhagia: a systematic review. *Br J Obstet Gynaecol* 2004; **111**: 734–40.

10 Trasi S, Pathare AV, Shetty S, Ghosh K, Salvi V, Mohanty D. The spectrum of bleeding disorders in women with menorrhagia; a report from Western India. *Ann Hematol* 2005; **84**: 339–42.

11 Chen YC, Chao TY, Cheng SN, Hu SH, Liu JY. Prevalence of von Willebrand disease in women with iron deficiency anaemia and menorrhagia in Taiwan. *Haemophilia* 2008; **14**: 768–74.

12 Bevan JA, Maloney KW, Hillery CA, Gill JC, Montgomery RR, Scott JP. Bleeding disorders: A common cause of menorrhagia in adolescents. *J Pediatr* 2001; **138**: 856–61.

13 Jayasinghe Y, Moore P, Donath S, Campbell J, Monagle P, Grover S. Bleeding disorders in teenagers presenting with menorrhagia. *Aust N Z J Obstet Gynaecol* 2005; **45**: 439–43.

14 Mikhail S, Varadarajan R, Kouides PA. The prevalence of disorders of haemostasis in adolescents with menorrhagia referred to a haemophilia treatment centre. *Haemophilia* 2007; **13**: 627–32.

15 Dilley A, Crudder S. von Willebrand disease in women: The need for recognition and understanding. *J Womens Health Gend Based Med* 1999; **8**: 443–5.

16 Kouides PA. Aspects of the laboratory identification of von Willebrand disease in women. *Semin Thromb Haemost* 2006; **32**: 480–4.

17 Blomback M, Eneroth P, Landgren BM, Lagerstrom M, Anderson O. On the intraindividual and gender variability of haemostatic components. *Thromb Haemost* 1992; **67**: 70–75.
18 Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. Variations in coagulation factors in women: effects of age, ethnicity, menstrual cycle and combined oral contraceptive. *Thromb Haemost* 1999; **82**: 1456–61.
19 Miller CH, Dilley A, Drews C, Richardson L, Evatt B. Changes in von Willebrand factor and Factor VIII levels during the menstrual cycle [letter]. *Thromb Haemost* 2002; **87**: 1082–3.
20 Onundarson PT, Gumundsdottir BR, Arnfinnsdottir AV, Kjeld M, Olafsson O. Von Willebrand factor does not vary during the normal menstrual cycle [letter]. *Thromb Haemost* 2001; **85**: 183–4.
21 Alperin JB. Estrogens and surgery in women with von Willebrand's disease. *Am J Med* 1982; **73**: 367–71.
22 Miller CH, Haff E, Platt SJ, *et al.* Measurement of von Willebrand factor activity: relative effects of ABO blood type and race. *J Thromb Haemost* 2003; **1**: 2191–7.
23 Higham JM, O'Brien PM, Shaw RW. Assessment of menstrual blood loss using a pictorial chart. *Br J Obstet Gynaecol* 1990; **97**: 734–9.
24 Kadir RA, Sabin CA, Pollard D, Lee CA, Economides DL. Quality of life during menstruation in patients with inherited bleeding disorders. *Haemophilia* 1998; **4**: 836–41.
25 Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. Frequency of inherited bleeding disorders in women with menorrhagia. *Lancet* 1998; **351**: 485–9.
26 Foster PA. The reproductive health of women with von Willebrand Disease unresponsive to DDAVP: results of an international survey. On behalf of the Subcommittee on von Willebrand Factor of the Scientific and Standardization Committee of the ISTH. *Thromb Haemost* 1995; **74**: 784–90.
27 James AH. More than menorrhagia: a review of the obstetric and gynaecological manifestations of bleeding disorders. *Haemophilia* 2005; **11**: 295–307.
28 Barr RD, Sek J, Horsman J, *et al.* Health status and health-related quality of life associated with von Willebrand disease. *Am J Hematol* 2003; **73**: 108–14.
29 Shankar M, Chi C, Kadir RA. Review of quality of life: menorrhagia in women with or without inherited bleeding disorders. *Haemophilia* 2008; **14**: 15–20.
30 Jarvis RR, Olsen ME. Type I von Willebrand's disease presenting as recurrent corpus hemorrhagicum. *Obstet Gynecol* 2002; 99: t–8.
31 Silwer J. Von Willebrand's disease in Sweden. *Acta Paediatr Scand* 1973; **238**: 1–159.
32 James AH, Kouides PA, Kadir RA, *et al.* Von Willebrand disease and other bleeding disorders in women experiencing menorrhagia or postpartum hemorrhage—Consensus on diagnosis and management from an international expert panel. *Am J Obstet Gynecol* 2009; **201**: 1–8.
33 Bonnar J, Sheppard BL. Treatment of menorrhagia during menstruation: randomised controlled trial of ethamsylate, mefenamic acid, and tranexamic acid [see comments]. *BMJ* 1996; **313**: 579–82.
34 Mohri H. High dose of tranexamic acid for treatment of severe menorrhagia in patients with von Willebrand disease. *J Thromb Thrombolysis* 2002; **14**: 255–7.
35 Ong YL, Hull DR, Mayne EE. Menorrhagia in von-Willebrand disease successfully treated with single daily dose tranexamic acid. *Haemophilia* 1998; **4**: 63–5.
36 Onundarson PT. Treatment of menorrhagia in von Willebrand's disease [letter]. *Haemophilia* 1999; **5**: 76.
37 Kadir RA, Lukes AS, Kouides PA, Fernandez H, Goudemand J. Management of excessive menstrual bleeding in women with hemostatic disorders. *Fertil Steril* 2005; **84**: 1352–9.
38 ClinicalTrials.gov. Efficacy and safety study of XP12B in women with menorrhagia, 2007. Available at: http://clinicaltrials.gov/ct/show/NCT00386308 (Accessed on 1 October 2009).
39 Rodeghiero F, Castaman G, Mannucci PM. Prospective multicenter study on subcutaneous concentrated desmopressin for home treatment of patients with von Willebrand disease and mild or moderate hemophilia A. *Thromb Haemost* 1996; **76**: 692–6.
40 Leissinger C, Becton D, Cornell C, Gill JC. High-dose DDAVP intranasal spray (Stimate) for the prevention and treatment of bleeding in patients with mild haemophilia A, mild or moderate type 1 von Willebrand disease and symptomatic carriers of haemophilia A. *Haemophilia* 2001; **7**: 258–66.
41 Kadir RA, Lee CA, Sabin CA, Pollard D, Economides DL. DDAVP nasal spray for treatment of menorrhagia in women with inherited bleeding disorders: a randomized placebo-controlled crossover study. *Haemophilia* 2002; **8**: 787–93.
42 Edlund M, Blomback M, Fried G. Desmopressin in the treatment of menorrhagia in women with no common coagulation factor deficiency but with prolonged bleeding time. *Blood Coagul Fibrinolysis* 2002; **13**: 225–31.
43 Kouides PA, Byams VR, Philipp CS, *et al.* A multisite management study of menorrhagia with abnormal laboratory hemostasis: a prospective crossover study of intranasal desmopressin and oral tranexamic acid. *Br J Haematol* 2009; **145**: 212–20.
44 Michiels JJ, van Vliet HH, Berneman Z, *et al.* Intravenous DDAVP and factor VIII–von Willebrand factor concentrate for the treatment and prophylaxis of bleedings in patients with von Willebrand disease type 1, 2 and 3. *Clin Appl Thromb Hemost* 2007; **13**: 14–34.
45 Berntorp E, Petrini P. Long-term prophylaxis in von Willebrand disease. *Blood Coagul Fibrinolysis* 2005; **16**(Suppl. 1): S23–6.
46 Berntorp E, Abshire T. The von Willebrand disease prophylaxis network: exploring a treatment concept. *J Thromb Haemost* 2006; **4**: 2511–12.
47 Nichols WL, Hultin MB, James AH, *et al.* von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA) 1. *Haemophilia* 2008; **14**: 171–232.
48 Kingman CEC, Kadir RA, Lee CA, Economides DL. The use of Levonorgestrel-releasing intrauterine system for the treatment of menorrhagia in women with inherited bleeding disorders. *Br J Obstet Gynaecol* 2004; **111**: 1425–8.
49 Chi C, Chase A, Kadir RA. Levonorgestrel-releasing intrauterine system for the management of menorrhagia in women with inherited bleeding disorders: long term follow-up. *Thromb Res* 2007; **119**(Suppl. 1): S101.
50 Lukes AS, Perry S, Ortel TL. von Willebrand's disease diagnosed after menorrhagia worsened from levonorgestrel intrauterine system. *Obstet Gynecol* 2005; **105**: 1223–6.
51 Stirling Y, Woolf L, North WR, Seghatchian MJ, Meade TW. Haemostasis in normal pregnancy. *Thromb Haemost* 1984; **52**: 176–82.
52 James AH, Jamison MG. Bleeding events and other complications during pregnancy and childbirth in women with von Willebrand disease. *J Thromb Haemost* 2007; **5**: 1165–9.
53 Sorosky J, Klatsky A, Norbert GF, Burchill RC. von Willebrand's disease complicating second trimester abortion. *Obstet Gynecol* 1980; **55**: 253–4.

54 Conti M, Mari D, Conti E, Muggiasca ML, Mannucci PM. Pregnancy in women with different types of von Willebrand disease. *Obstet Gynecol* 1986; **68**: 282–5.
55 Caliezi C, Tsakiris DA, Behringer H, Kuhne T, Marbet GA. Two consecutive pregnancies and deliveries in a patient with von Willebrand's disease type 3. *Haemophilia* 1998; **4**: 845–9.
56 Chediak JR, Alban GM, Maxey B. von Willebrand's disease and pregnancy: management during delivery and outcome of offspring. *Am J Obstet Gynecol* 1986; **155**: 618–24.
57 Mannucci PM. How I treat patients with von Willebrand disease [Review]. *Blood* 2001; **97**: 1915–19.
58 Sanchez-Luceros A, Meschengieser SS, Turdo K, *et al.* Evaluation of the clinical safety of desmopressin during pregnancy in women with a low plasmatic von Willebrand factor level and bleeding history. *Thromb Res* 2007; **120**: 387–90.
59 As AK, Hagen P, Webb JB. Tranexamic acid in the management of postpartum haemorrhage. *Br J Obstet Gynaecol* 1996; **103**: 1250–1.
60 Salman MC, Cil B, Esin S, Deren O. Late postpartum hemorrhage due to von Willebrand disease managed with uterine artery embolization. *Obstet Gynecol* 2008; **111**: 573–5.
61 Chi C, Lee CA, England A, Hingorani J, Paintsil J, Kadir RA. Obstetric analgesia and anaesthesia in women with inherited bleeding disorders. *Thromb Haemost* 2009; **101**: 1104–11.
62 Varughese J, Cohen AJ. Experience with epidural anaesthesia in pregnant women with von Willebrand disease. *Haemophilia* 2007; **13**: 730–3.
63 Marrache D, Mercier FJ, Boyer-Neumann C, Roger-Christoph S, Benhamou D. Epidural analgesia for parturients with type 1 von Willebrand disease. *Int J Obstet Anesth* 2007; **16**: 231–5.
64 Mathew P, Greist A, Maahs JA, Lichtenberg EC, Shapiro AD. Type 2B vWD: the varied clinical manifestations in two kindreds. *Haemophilia* 2003; **9**: 137–44.
65 Dennis MW, Clough V, Toh CH. Unexpected presentation of type 2N von Willebrand disease in pregnancy. *Haemophilia* 2000; **6**: 696–7.
66 Rick ME, Williams SB, Sacher RA, McKeown LP. Thrombocytopenia associated with pregnancy in a patient with type IIB von Willebrand's disease. *Blood* 1987; **69**: 786–9.

47 Treatment of von Willebrand disease: desmopressin

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Introduction

In von Willebrand disease (VWD), the principle of treatment or prevention of bleeding is the transient correction of the dual plasma deficiency of von Willebrand factor (VWF) and factor VIII (FVIII). The latter deficiency is secondary to that of VWF, its carrier and stabilizer in plasma [1]. Correction of both deficiencies can be achieved by administering the synthetic peptide desmopressin (DDAVP) or, in patients unresponsive to this agent, plasma or plasma fractions.

Desmopressin (DDAVP)

This peptide is an analog of the antidiuretic hormone vasopressin devoid of V1 agonist effects. Accordingly, its administration is not accompanied by such adverse effects as vasoconstriction, hypertension, uterus contraction, and colicky abdominal pain. Being a V2 agonist, the drug retains the antidiuretic activity of vasopressin. Desmopressin raises VWF by secreting this moiety into plasma from its natural site of synthesis and storage, the vascular endothelial cell [2]. It is not well established how desmopressin raises FVIII in parallel with VWF.

Factor VIII and VWF increase three- to fivefold above baseline values when desmopressin is infused intravenously [3,4] (the most frequently used route of administration), subcutaneously [5], or intranasally [4] (the last two routes used mainly for self-treatment at home). The recommended dosages are 0.3 μg/kg by slow intravenous infusion or subcutaneous injection, and fixed doses of 300 μg in adults and 150 μg in children by intranasal spray. Lower and higher doses are less effective and no more effective, respectively. The factor-raising effect of desmopressin is present in normal individuals as well as in patients with mild hemophilia and von Willebrand disease, except for those with undetectable levels of the factors (patients with severe hemophilia and with type 3 von Willebrand disease) [4].

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Clinical use

These properties of desmopressin have been exploited therapeutically to treat patients with VWD (and mild hemophilia) at the time of bleeding or before invasive surgical procedures [6–8]. The likelihood of an efficacious hemostatic response should be assessed with a test dose given to candidates for treatment either at the time of diagnosis or when an elective treatment is planned [9]. Table 47.1 shows the schedule of desmopressin administration and blood sampling recommended to evaluate the degree of laboratory response to a test dose. On the basis of the results obtained in this manner, caregivers can evaluate whether or not the attained factor levels and the predicted duration of their persistence in plasma are of such a degree that the successful management of any given clinical situation can be predicted (Table 47.2). In practical terms, patients with FVIII and VWF levels of 10–20% or more are the most likely to benefit from the therapeutic use of desmopressin. For instance, a patient with baseline levels of 5% should reach peak postinfusion levels of 15–20% (which may be sufficient to stop a hemarthrosis but not to handle dental extractions). On the other hand, a patient with baseline levels of 20–25% may reach levels as high as 50–70%, adequate to provide hemostasis during dental extractions. Major surgical procedures can be successfully carried out in patients with levels of 30–40%, because postdesmopressin levels in excess of 100% are usually reached.

Responses to desmopressin can also be predicted from knowledge of the different phenotypes of VWD. Type 1, the most frequent phenotype, accounting for 60–80% of cases and caused by the quantitative deficiency of VWF and of FVIII, is the most responsive to desmopressin [7–10]. Type 2, accounting for 20–30% of cases and caused by a dysfunctional VWF protein synthesized in normal amounts, is generally poorly responsive to desmopressin, because the compound triggers the secretion into plasma of a dysfunctional moiety [7,8,11]. Exceptions to this general rule are some patients with the subtypes 2N, in whom FVIII increases adequately [8,12]. Type 3 VWD, the most severe form, accounting for 2–5% of cases, is almost invariably unresponsive to desmopressin, because affected patients lack secretable VWF.

A limitation of DDAVP is a progressive decrease in the degree of factor rise observed in patients with VWD (and mild

Table 47.1 Schedule for the test dose of desmopressin to assess responsiveness in patients with von Willebrand disease (and mild hemophilia).

Step 1 Infuse over 30 min 0.3 μg/kg desmopressin in 100 mL saline in newly diagnosed patients or in those who must undergo an elective treatment
Step 2 Obtain citrated blood samples 60 min after starting desmopressin (postinfusion peak) and at 4 h (to assess the rate of factor clearance)
Step 3 Measure factor VIII coagulant activity and ristocetin cofactor or collagen-binding activity

If the subcutaneous or intranasal routes are preferred for desmopressin administration, the same schedule should be followed.

Table 47.2 Target levels of factor VIII (FVIII) and von Willebrand factor recommended in clinical situations for patients with von Willebrand disease.

Clinical situation	Target
Major surgery	Peak FVIII levels[a] of 100% and trough daily levels of at least 50% until healing is complete (usually 5–10 days)
Minor surgery	Peak FVIII levels of 60% and trough daily levels of at least 30% until healing is complete (usually 2–4 days)
Dental extractions	Peak FVIII levels of 60% (single dose)
Spontaneous bleeding episodes	Peak FVIII levels higher than 50% until bleeding stops (usually 2–4 days)
Delivery and puerperium[a]	Peak FVIII levels higher than 80% and trough levels of at least 30%, usually for 3–4 days

[a]For those who prefer to monitor and measure von Willebrand factor, the same target levels of ristocetin cofactor or collagen binding activity are recommended.

hemophilia) treated repeatedly, a phenomenon known as tachyphylaxis [13]. The only way to ascertain whether and when tachyphylaxis develops is to measure FVIII and VWF levels in plasma after each desmopressin infusion. Depending on the peak factor levels attained post infusion and on trough levels, the care-giver can decide whether treatment can be safely stopped because rebleeding is unlikely to occur, or whether it is necessary to revert to the infusion of plasma fractions.

The obvious advantages of desmopressin are the absent risk of transmission of blood-borne infections and its relatively low cost. For this reason, desmopressin is the treatment of choice in responsive patients with VWD (and mild hemophilia). Desmopressin is listed by the World Health Organization (WHO) among essential drugs. However, not all countries have implemented WHO recommendations, and in many of them desmopressin is not available or is licensed only for the other main clinical indications of the compound, i.e., diabetes insipidus and nocturnal enuresis.

Monitoring treatment

The purpose of monitoring desmopressin treatment with laboratory testing is to establish whether or not the degree of correction over time of the FVIII and VWF defects is adequate to control bleeding, spontaneous or postoperative (Table 47.2). For minor bleeding episodes and invasive procedures such as dental extractions, monitoring is usually not necessary, because the hemostatic response is quite predictable if the dosages recommended above are used. For more severe bleeding episodes and major surgery, monitoring is usually necessary to establish whether or not the occurrence of tachyphylaxis has rendered the patient unresponsive.

FVIII assays are the tests of choice for monitoring treatment in patients with VWD. VWF measurements such as ristocetin cofactor and collagen binding assays can also be used, but they are more technically demanding and less standardized than FVIII assays, more costly, and difficult to set up. Moreover, there is much less experience (compared with monitoring of FVIII) on peak and trough VWF levels needed to reach and maintain hemostasis (Table 47.2). If one chooses to monitor patients with VWF assays, the same peak and trough levels recommended for FVIII are tentatively recommended (Table 47.2).

It is not usually necessary to monitor the skin bleeding time, not only because this test is difficult to standardize and has poor reproducibility, but mainly because it is a poor predictor of hemostasis during soft-tissue and postoperative bleeding. There is evidence, for instance, that surgical hemostasis is reached and maintained by desmopressin as well as by plasma fractions even if the bleeding time is prolonged, provided sufficient levels of plasma FVIII are reached. It is also unnecessary to evaluate the post-treatment multimeric pattern of VWF, whereas knowledge of this pattern is necessary to establish the phenotype of VWD and to decide the optimal treatment with desmopressin or plasma fractions.

Side-effects

Transient headache, facial flushing, and mild tachycardia are relatively frequent side-effects, usually well tolerated by patients. The antidiuretic effect is not perceived clinically in patients with a normal capacity to excrete water, if the drug is given at the recommended time intervals (every 24 h) and fluid intake is not excessive. Laboratory monitoring of osmolality and electrolytes is not necessary, but body weighing is a recommended simple and inexpensive precautionary measure. Severe symptoms owing to water intoxication, such as cerebral edema and seizures, are rarely reported, more often in infants and young children [14] but sometimes also in adults. There are occasional reports of arterial thrombosis during treatment [15,16], so that the drug should be avoided in patients with overt cardiovascular disease. DDAVP can be safely used in pregnant women, because it is devoid of oxytocic properties. Theoretically, there is some risk for hyponatremia

for the fetus when DDAVP is administered in the last trimester of pregnancy, particularly just prior to parturition.

Adjuvant treatments

Treatment with desmopressin is usually given in association with antifibrinolytic amino acids. ε-Aminocaproic acid and tranexamic acid are synthetic compounds that inhibit fibrinolysis by saturating the binding sites on plasminogen, thereby impeding plasmin formation. ε-Aminocaproic acid can be administered orally, intravenously, or topically at doses of 60 mg/kg every 6 h, tranexamic acid by the same routes at doses of 15 mg/kg every 8 h. In general, the effectiveness of these compounds in the treatment of bleeding disorders is explained by the role of local hyperfibrinolysis in the onset and maintenance of bleeding in such mucosal tracts as the nasopharynx and the gastrointestinal and genitourinary tracts. Sometimes in these situations, antifibrinolytic drugs are sufficient to stop bleeding without the need to revert to desmopressin or plasma products. More often, they are given as adjuvants, because they help to reduce the total amount of factors by stabilizing the formed fibrin clots. A typical example is dental surgery, in which these drugs can also be used locally, as mouthwashes. We recommend the use of antifibrinolytic amino acids together with desmopressin for the previously mentioned reasons. Even though desmopressin induces a brisk, short-term increase of tissue plasminogen activation, there is no evidence that this effect affects hemostasis in treated patients.

Conclusion

The therapeutic use of desmopressin in VWD (and mild hemophilia) has now withstood the experience of more than a quarter of a century. There is no doubt that the use of this compound at a time when plasma concentrates were not virus inactivated spared many patients with mild hemophilia and VWD from blood-borne infections and the related ominous consequences [17], which were felt to be particularly dramatic in patients with mild bleeding disorders, who need treatment much less frequently than those with severe disease. The advent of virus-inactivated plasma concentrates and the availability of recombinant factors in hemophilia have currently rendered less crucial the safety afforded by a synthetic drug such as desmopressin. Hence its main appeal is its relatively low cost, particularly for developing countries. It is baffling that, despite its early inclusion in the WHO-recommended list of essential drugs, desmopressin is not licensed or available in many developing countries. For instance, it was hardly used until recently in the Islamic Republic of Iran, a country with good levels of hemophilia care delivery.

In terms of new nontransfusional treatments of hemophilia and VWD, it has been shown that the cytokine interleukin 11 leads to a gradual and sustained increase in FVIII and VWF in mice and dogs, different from the short-lasting effect elicited by desmopressin [18–20]. One might envision the use of desmopressin when a short-term increase in FVIII and VWF is needed (treatment of acute bleeding) and of interleukin 11 when a longer duration of the hemostatic effect is needed (management of major surgery).

References

1 Sadler JE. Biochemistry and genetics of von Willebrand factor. *Annu Rev Biochem* 1998; **67**: 395–424.

2 Kaufmann JE, Oksche A, Wollheim CB, *et al.* Vasopressin induced von Willebrand factor secretion from endothelial cells involves V2 receptors and cAMP. *J Clin Invest* 2000; **106**: 107–16.

3 Mannucci PM, Aberg M, Nilsson IM, Robertson B. Mechanism of plasminogen activator and factor VIII increase after vasoactive drugs. *Br J Haematol* 1975; **30**: 81–93.

4 Mannucci PM, Canciani MT, Rota L, Donovan BS. Response of factor VIII/von Willebrand factor to DDAVP in healthy subjects and patients with haemophilia A and von Willebrand disease. *Br J Haematol* 1981; **47**: 283–93.

5 Rodeghiero F, Castaman G, Mannucci PM. Prospective multicenter study on subcutaneous concentrated desmopressin for home treatment of patients with von Willebrand disease and mild or moderate hemophilia A. *Thromb Haemost* 1996; **76**: 692–6.

6 Mannucci PM, Ruggeri ZM, Pareti FI, Capitanio AM. Deamino-8-D-arginine vasopressin: a new pharmacological approach to the management of haemophilia and von Willebrand disease. *Lancet* 1977; **1**: 869–72.

7 Revel-Vilk S, Schmugge M, Carcao MD, *et al.* Desmopressin (DDAVP) responsiveness in children with von Willebrand disease. *J Pediatr Hematol Oncol* 2003; **25**: 874–9.

8 Federici AB, Mazurier C, Berntorp E, *et al.* Biological response to desmopressin in patients with severe type 1 and type 2 von Willebrand disease. Results of a Multicenter European Study. *Blood* 2004; **103**: 2032–8.

9 Rodeghiero F, Castaman G, Di Bona E, Ruggeri M. Consistency of responses to repeated DDAVP infusions in patients with von Willebrand's disease and hemophilia A. *Blood* 1989; **74**: 1997–2000.

10 Mannucci PM, Lombardi R, Bader R, *et al.* Heterogeneity of type I von Willebrand disease: evidence for a subgroup with an abnormal von Willebrand factor. *Blood* 1985; **66**: 796–802.

11 Ruggeri ZM, Mannucci PM, Lombardi R, *et al.* Multimeric composition of factor VIII/von Willebrand factor following administration of DDAVP: implications for pathophysiology and therapy of von Willebrand's disease subtypes. *Blood* 1982; **59**: 1272–8.

12 Mazurier C, Gaucher C, Jorieux S, Goudemand M, and the Collaborative Group. Biological effect of desmopressin in eight patients with type 2 N ("Normandy") von Willebrand disease. *Br J Haematol* 1994; **88**: 849–54.

13 Mannucci PM, Bettega D, Cattaneo M. Patterns of development of tachyphylaxis in patients with hemophilia and von Willebrand disease after repeated doses of desmopressin (DDAVP). *Br J Haematol* 1992; **82**: 87–93.

14 Smith TJ, Gill JC, Ambroso DR, Hathaway WE. Hyponatremia and seizures in young children given DDAVP. *Am J Hematol* 1989; **31**: 199–202.

15 Bond L, Bevin D. Myocardial infarction in a patient with hemophilia A treated with DDAVP. *N Engl J Med* 1988; **318**: 121 (letter).
16 Byrnes JJ, Larcada A, Moake JL. Thrombosis following desmopressin for uremic bleeding. *Am J Hematol* 1988; **28**: 63–5.
17 Mannucci PM, Ghirardini A. Desmopressin: twenty years after. *Thromb Haemost* 1997; **78**: 958.
18 Denis CV, Kwack K, Saffaripour S, *et al*. Interleukin 11 significantly increases plasma von Willebrand factor and factor VIII in wild type and von Willebrand disease mouse models. *Blood* 2001; **97**: 465–72.
19 Olsen EH, McCain AS, Merricks EP, *et al*. Comparative response of plasma vWF in dogs to upregulation of vWF mRNA by interleukin-11 versus Weibel-Palade body release by desmopressin (DDAVP). *Blood* 2003; **102**: 436–41.
20 Ragni MV, Jankowitz RC, Chapman HL, Merricks EP, Kloos MT, Dillow AM, Nichols TC. A phase II prospective open-label escalating dose trial of recombinant interleukin-11 in mild von Willebrand disease. *Haemophilia* 2008; **14**: 968–77.

48 Treatment of von Willebrand disease: therapeutic concentrates

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Introduction

The treatment or prevention of bleeding in patients with von Willebrand disease (VWD) requires correction of the defects in platelet–vessel wall interactions and in secondary hemostasis caused by a deficiency of factor VIII (FVIII). These defects vary between patients and subtypes of VWD. The two hemostatic actions of von Willebrand factor (VWF) are important in different clinical situations; platelet–vessel wall interactions are more significant in microvascular bleeding, particularly from mucosal surfaces, and FVIII deficiency for the control of deep tissue bleeding and promoting wound healing. The role of antifibrinolytic drugs is important in clinical situations where mucosal bleeding or increased fibrinolytic activity is likely.

The optimal hemostatic treatment of VWD is with desmopressin (DDAVP) [1] because pooled plasma-derived VWF containing concentrates are associated with the potential risk of transmission of infectious agents. However, not all patients with VWD are suitable for treatment with DDAVP. In these patients VWF-containing concentrates are indicated and will be the focus of this chapter. Patients who may need to be treated with pooled blood products should be vaccinated against hepatitis A and B [2].

Data relating to the properties of VWF-containing concentrates in *in vitro* tests and hemostatic effects *in vivo* is increasing. Concentrate use, however, is complicated by the fact that correction of VWF levels in the plasma may not reproduce the physiologic action of VWF at the vessel wall under high shear. When making therapeutic decisions, therefore, consideration should be given to studies that demonstrate clinical hemostatic efficacy rather than correction of laboratory parameters [3–5].

Indications for the use of von Willebrand factor-containing concentrates

von Willebrand factor-containing concentrates are indicated for VWD patients unresponsive or unsuitable for DDAVP. Many clinicians, for example, are cautious about treating young children with DDAVP because of the risk of hyponatremic seizures [6], though this risk must be balanced against exposing a young child to a pooled blood product. Atherosclerosis is a contraindication to DDAVP because of the risk of arterial thrombosis [7,8] and venous thromboembolism (VTE) has also been noted [9–11]. Some clinicians recommend an upper age limit for DDAVP use, although there is no clear consensus. The VWD subtype affects the decision on whether to use DDAVP or a VWF-containing concentrate (Table 48.1).

Type 3 von Willebrand disease

Patients with type 3 VWD must be treated with a VWF-containing concentrate because they will not respond to DDAVP.

Type 2B von Willebrand disease

Many clinicians recommend that DDAVP not be used in type 2B VWD because of the risk of thrombocytopenia [12]. There are a few reports, however, where DDAVP has been used safely [13,14].

Type 2A and type 2M

The response to DDAVP in patients with type 2A or 2M VWD is unpredictable and a therapeutic trial should assess the response of VWF ristocetin cofactor (VWF:RCo) and FVIII [15,16]. Patients shown not to achieve adequate levels of either protein require treatment with a VWF-containing concentrate. This is particularly true for major operative procedures where therapeutic levels of VWF and FVIII must be maintained for a prolonged period.

Type 2N von Willebrand disease

DDAVP increases FVIII to normal in most patients but the half-life is significantly reduced [17]. Minor bleeds and procedures can often be treated with DDAVP. Major bleeds or procedures that require a prolonged correction of FVIII are likely to require treatment with a VWF-containing concentrate which normalizes the half-life and plasma level of endogenous FVIII [18].

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Table 48.1 Indications for the use of a VWF-containing concentrate to treat von Willebrand disease (VWD).

Definite	Relative
Type 3 VWD	Age less than 2 years
Type 2B VWD[a]	Older age[b]
Inadequate response to DDAVP	Type 2A and 2M VWD[c]
Atherosclerotic disease	Heart failure and patients on diuretics
	Type 2N VWD[c]
	Acquired VWD

[a]There are reports of DDAVP being used safely in patients with type 2B VWD.
[b]There is a lack of consensus on an upper age limit and many clinicians do not regard age alone as a contraindication to DDAVP.
[c]Some patients may respond to DDAVP for minor procedures and bleeding episodes.

Type 1 von Willebrand disease

Most patients with type 1 VWD respond to DDAVP [1,19]. Some patients do not respond adequately, particularly if they lack platelet VWF [20]. Some patients have very good initial responses to DDAVP but the half-life of the released VWF and FVIII is short. This is particularly associated with VWD Vicenza (R1205H) but has also been seen with other mutations [21,22]. A DDAVP trial should include a 3–4-h time point to assess this [5]. For major surgery it may not be possible to sustain adequate VWF and FVIII levels with DDAVP and patients may need a VWF-containing concentrate.

General properties required of a von Willebrand factor-containing concentrate

Concentrates available for the treatment of VWD may contain both FVIII and VWF or may contain very high-purity VWF (VHP-VWF) with minimal or no FVIII. The important considerations when choosing a concentrate are summarized in Table 48.2. The *in vitro* and *in vivo* characteristics of many VWF-containing concentrates have been comprehensively reviewed [23]. Important progress in standardization of VWF measurements in both plasma samples and concentrates has been facilitated through the calibration of the WHO first international standard for VWF:Ag and VWF:RCo [24].

It is often stated that the presence of high-molecular-weight multimers (HMWM) is essential for the *in vivo* correction of the primary hemostatic defect. The amount of HMWM that is required for effective hemostasis remains controversial [23]. There are no data that compare multimer composition or other laboratory parameters of VWF-containing concentrates with hemostatic efficacy. No concentrate normalizes VWF multimers *in vivo* [25] and cryoprecipitate, with a full complement of HMWM, does not consistently correct the bleeding time [26]. In practice, however, many VWF-containing concentrates, without a full complement of HMWM, have established clinical efficacy, implying that consideration of this property alone oversimplifies the clinical efficacy assessment of a VWF-containing concentrate [18,27–42].

Table 48.2 Important considerations when choosing a VWF-containing concentrate.

Viral inactivation steps and proven safety for transmission of infectious agents
Hemostatic efficacy for all subtypes of VWD demonstrated in clinical trials
Potency labeling for VWF:RCo and factor VIII
Established pharmacokinetics
Plasma source
Side-effect profile
Licensed within country of use
Availability

Regulatory agencies that assess data about the pathogen safety of VWF-containing concentrates indicate that these products appear to be at least as free of pathogen risk as current plasma-derived products used for hemophilia A. Further, assessments of clinical efficacy indicate that products containing VWF are efficacious in promoting platelet adhesion and stabilizing factor VIII. These conclusions are based on each product having been tested for pharmacokinetic properties and efficacy in all subtypes of VWD and being labeled with both VWF:RCo and factor VIII potency [43].

Clinical studies of von Willebrand factor-containing concentrates

Many studies have been published that demonstrate hemostatic efficacy of a number of VWF-containing concentrates in the clinical settings of acute bleeds and surgical procedures [3–5,18,27,28,30–35,37,39,41,42,44–48]. There are no data to support the view that any concentrate has superior hemostatic efficacy to any other, although the quantity of safety and efficacy data varies markedly depending on how long a concentrate has been available in the market. The availability of each VWF-containing concentrate will vary between countries. Commonly used products and the studies demonstrating their pharmacokinetic properties are shown in Table 48.3 [1,49–52].

Clinical management

General principles

The amount of VWF-containing concentrate required to correct the hemostatic defect in VWD is dependent on the

Table 48.3 Characteristics of some commonly used VWF-containing concentrates.

Product and manufacturer	Plasma source	Manufacturing process	Viral inactivation	VWF:RCo/ FVIII ratio	*In vivo* recovery FVIII IU/dL per IU/kg	*In vivo* recovery VWF:RCo IU/dL per IU/kg	Reference
Alphanate (Alpha)	USA	Heparin ligand chromatography	SD and dry heat (80 °C for 72 h)	1.6:1	2.1 ± 0.4 (mean ± SD)	2.9 ± 1.3 (mean ± SD)	[39]
Biostate	Australia, New Zealand, Malaysia, Singapore, Hong Kong, USA	Heparin/glycine precipitation, gel filtration, chromatography	SD and dry heat (80 °C for 72 h)	2.1:1	1.1 (0.88–1.32) (mean 90% CI)	0.85 (0.77–0.92) (mean 90% CI)	[50]
Factor 8Y (Bio Products Laboratory)	USA	Glycine/NaCl treatment of cryoprecipitate	Dry heat (80 °C for 72 h)	2:1	Type 3: 2.4 ± 1.1; type 1: 5.0 ± 4.5 (mean ± SD)	Type 3: 1.4 ± 0.05; type 2A: 2.0 ± 0.06; type 1: 2.3 ± 0.5 (mean ± SD)	[52]
Fandi (Grifols)	USA	Heparin ligand chromatography	SD and dry heat (80 °C for 72 h)	1.6:1	No data	No data	[33]
Haemate-P (Aventis Berhing)	Germany, Austria, USA	Glycine/NaCl treatment of cryoprecipitate	Pasteurized (60 °C for 10 h)	2.5:1	2.7 (1.9–3.7) median (range)	2.1 (1.1–2.7) median (range)	[49]
Immunate (Baxter)	USA, Austria, Sweden, Germany, Czech Republic	Ion exchange chromatography	SD and vapor heat (60 °C for 10 h)		1.9 (0.8–3.5) mean (range)	1.8 (0.4–3.6) mean (range)	[28]
Wilate (Octapharma)	Austria, Sweden, Switzerland, Germany, USA	Ion exchange chromatography and Ultra/Dia filtration	SD and terminal dry heat (100 °C for 120 min)	1:1			
Wilfactin (LFB)	France, Germany, Switzerland	Ion and affinity-exchange chromatography	SD and dry heat (80 °C for 72 h); nanofiltration (35 nM)	10:1	5.8 ± 1 IU/dL (FVIII synthesis rate)	2.1 ± 0.3	[51]

These concentrates may not be licensed in all countries.

type of bleed or surgery, the patient's baseline VWF:RCo and FVIII and the subtype of VWD. Different types of surgery necessitate different target VWF:RCo and FVIII levels (Table 48.4) [5]. Patients also vary in their response to infusions of VWF-containing concentrates. Therefore, treatment regimens need to be individualized. Knowledge of an individual's pharmacokinetic response to infused VWF-containing concentrate is a therapeutic advantage and this previous demonstration of hemostatic efficacy has correlated well with prospective therapy for surgery or management of acute bleeding episodes [45]. However, VWF:RCo levels in the plasma do not necessarily predict correction of the platelet/vessel wall defect *in vivo* and despite normalization of laboratory parameters, close clinical observation for bleeding is necessary.

Table 48.4 Target laboratory parameters for invasive procedures.

	Target VWF:RCo	Target FVIII
Major		
Time of procedure	80–100 IU/dL	100 IU/dL
Subsequent	Maintain trough above 50 IU/dL until hemostasis secure	Maintain trough above 50 IU/dL until wound healing complete
Minor		
Time of procedure	30–50 IU/dL	50 IU/dL
Subsequent	Unlikely to be important	Maintain trough of 50 IU/dL until wound healing complete

Many clinicians adjust the initial dose of VWF-containing concentrates based on the individual patient's baseline levels. Subsequent dosing is then calculated using recovery and half-life measurements [5,30]. It is important to note that infusion of a VWF-containing concentrate increases FVIII *in vivo* both from the addition of the infused FVIII in the concentrate *and* by stabilizing endogenous FVIII [23].

It is becoming common practice to dose patients using the labeled VWF:RCo content in the concentrate chosen for treatment or prophylaxis. This has been shown to be effective in both a prospective study [39] and in retrospective studies involving both children and adults [36,53]. The VWF:RCo and FVIII levels required for hemostasis have not been established in clinical trials. However, by consensus for major operative procedures or to treat a significant bleeding the VWF:RCo and FVIII should be raised to above 80 IU/dL and maintained above 50 IU/dL until hemostasis is secured. The FVIII level should be maintained above 50 IU/dL until wound healing is complete. Minor surgery may be performed successfully with a VWF:RCo and FVIII of about 50 IU/dL [3,5,53,54].

Some clinicians recommend fixed initial doses of VWF-containing concentrate irrespective of the patient's baseline levels. In a prospective study using Alphanate®, bleeding episodes were treated with 40 IU/kg VWF:RCo (50 IU/kg for children) and surgical prophylactic dosing was 60 IU/kg VWF:RCo (75 IU/kg in children) [39]. Italian guidelines suggest an initial dose of 50 IU/kg for major surgery, 30 IU/kg for minor surgery and 20 IU/kg for dental surgery [3].

Dosing of VHP-VWF concentrate differs from other concentrates because the deficiency of FVIII is corrected over 6–12 h by stabilization of endogenous FVIII. When rapid correction of hemostasis is required, an initial dose of factor VIII must also be infused [18,37,51]. Combined data from a number of trials for a VHP-VWF concentrate (Wilfactin®) has been published: clinical outcomes were excellent/good in 89% of spontaneous bleeds, although 38% of cases needed concomitant FVIII. Excellent/good efficacy was also observed in 95 elective surgical procedures without the need for prophylactic therapy with FVIII. Yet, in another 13 urgent surgeries FVIII was required [48].

Treatment of acute bleeds

For severe spontaneous or trauma-induced bleeding the VWF:RCo and factor VIII should be increased to approximately 80 IU/dL until bleeding has been controlled. Further treatment may be required to prevent recurrence of the bleeding. Antifibrinolytic agents are likely to be useful in mucosal bleeding. Local measures may also be required to control severe epistaxis or gastrointestinal bleeding. Lower levels of VWF:RCo and factor VIII are required for minor bleeds [3,5]. VHP-VWF is administered at a dose of 50–60 U/kg VWF:RCo and at least 30–40 IU/kg FVIII if the individual's steady-state FVIII is less than 20 IU/dL or if the bleeding is severe [48].

Prophylaxis

Retrospective data from 35 patients on long-term prophylaxis demonstrate a significant reduction in mucosal bleeding and hemarthroses and no evidence of progression of arthropathy. This suggests a potential role for this treatment modality for prophylactic infusions [55]. Such prophylactic treatment has been reported in a further 11 patients; seven treated for mucosal bleeding and four for hemarthroses. Bleeding was prevented in 8 of the 11 patients and reduced in three [34]. Prophylaxis with VHP-VWF concentrate (50 U/kg 2–3 times a week) has been reported in four patients in which treatment appeared to reduce the risk of spontaneous bleeding for 48 h following infusion [48].

The use of VWF-containing concentrates for prophylactic treatment has increased over the last few years and in some regions appears to be relatively common. According to a recent survey of 74 centers in Europe and North America [56] most patients on prophylaxis have type 3 VWD (74.5%) with 17.6% type 2 and 7.8% type 1. In Europe 28.7% and North America 12.2% of patients with type 3 disease were being treated on a prophylactic regimen. Indications for prophylaxis were hemarthroses (40%), epistaxis/oral (23%), gastrointestinal bleeding (14%), and menorrhagia (5%).

The optimum prophylactic regimen has not been established and it is plausible that treatment does not need to be administered as often as for severe hemophilia. A randomized study comparing on-demand and prophylactic treatment (60 U VWF:RCo every second or third day) in 24 patients over a 12-month period is ongoing [34]. In addition, an escalation study has been initiated starting with one infusion of 50 U/kg VWF:RCo per week building up to two and three infusions if breakthrough bleeds occur. For women with menorrhagia the initial dose will be infused on day 1, escalating to recurrent dosing on days 2 and 3 if significant bleeding continues [56].

The results of these studies will help to better define the role of prophylaxis in VWD and give data on the optimum regimen. However, until the results are available, patients with recurrent joint bleeds or mucosal bleeds interfering with day-to-day life may benefit from being treated on a prophylaxis regimen.

Management of surgery

General considerations

Patients with VWD who undergo surgical procedures need regular clinical assessment of hemostasis. It is important to recognize that abnormal bleeding may be the result of surgical bleeding rather than inadequate hemostasis. Patients who bleed despite apparently adequate treatment should have a full blood count, coagulation screen, and FVIII and VWF:RCo levels measured urgently. A bleeding time may occasionally be useful, although many coagulation experts eschew this measurement for inadequate sensitivity and specificity. In patients

with type 3 VWD the development of an inhibitor to VWF occurs rarely, but screening for this complication is prudent [5]. The indication for use of a VWF-containing concentrate in managing surgery depends, in part, on the subtype of the patient's VWD.

Type 1 von Willebrand disease

von Willebrand factor-containing concentrates are more likely to be needed for larger operations or in situations in which prolonged correction of the hemostatic defect is required. In type 1 VWD the VWF and FVIII levels often increase temporarily post operatively because of an acute phase response.

Type 2A or type 2M von Willebrand disease

Minor procedures can be performed after DDAVP treatment in some patients but major procedures are likely to require a VWF-containing concentrate. Correcting the VWF:RCo level may lead to excessively high FVIII levels, thereby increasing the risk of venous thromboembolism (VTE).

Type 2B von Willebrand disease

Most clinicians recommend VWF-containing concentrates to cover invasive procedures in type 2B VWD.

Type 2N von Willebrand disease

Minor procedures may be done with DDAVP. A VWF-containing concentrate normalizes the half-life of endogenous FVIII. Raising FVIII to about 100 IU/dL perioperatively and maintaining it at *in vivo* levels exceeding 50 IU/dL until wound healing is complete provides adequate hemostatic prophylaxis for such procedures. Infusion of a VHP-VWF concentrate will also lead to normalization of the FVIII level over 6–12 h [17,18]. The improved half-life of FVIII means that once-daily treatment is usually sufficient.

Type 3 von Willebrand disease

Patients with type 3 VWD who are undergoing invasive procedures must be treated with a VWF-containing concentrate.

Patients who have alloantibodies to VWF are at risk of anaphylactic reactions with the use of VWF-containing concentrates [57]. Experience in managing these patients is limited; however, good hemostasis has been reported with the use of high doses or infusions of recombinant factor VIII [3]. Recombinant FVIIa has also been used [58].

Acquired von Willebrand disease

Some patients with acquired VWD who have failed to respond to DDAVP have responded to VWF-containing concentrates [59].

Minor procedures

In general, a VWF:RCo and FVIII of 50 IU/dL should be adequate for a minor procedure and often only one infusion is required.

Dental treatment

For dental extraction or inferior dental nerve block, in patients unsuitable for DDAVP, treatment with a VWF-containing concentrate is indicated. Many clinicians aim to increase the VWF:RCo and FVIII to about 50 IU/dL with a single infusion of a VWF-containing concentrate. An antifibrinolytic agent should be given for 7–10 days following a dental extraction.

Major procedures

An infusion of VWF:RCo-containing concentrate calculated to increase the VWF:RCo level to 80–100 IU/dL at the time of surgery is recommended. The level should be maintained above 50 IU/dL until hemostasis is secure [5,30]. The FVIII should be raised to about 100 IU/dL perioperatively and maintained above 50 IU/dL until would healing is complete. This requires regular monitoring of both VWF:RCo and FVIII.

VHP-VWF concentrates are infused at a dose of 50 IU/kg VWF:RCo 12–24 h before surgery if the FVIII is less than 60 IU/dL. A further dose is given 1 h before surgery and subsequent infusions are given to maintain a VWF:RCo level > 60 U/dL and a FVIII level > 40 IU/dL. Unscheduled surgery is treated with VHP-VWF concentrate at 50 U/kg and FVIII to the same target levels [48]. Continuous infusion of VWF/FVIII or VHP-VWF concentrate has also been successfully used to provide hemostatic prophylaxis for major surgery [38,60].

The risk of VTE should be assessed in patients undergoing major surgery who receive VWF-containing concentrates. High levels of FVIII should be avoided and venous thrombophylaxis considered [10,11].

Venous thrombosis

Venous thrombosis has been associated with the use of intermediate-purity VWF-containing concentrates [10,11,30]. A review of the literature found that patients usually have congenital or acquired risk factors contributing to VTE [35]. High FVIII levels associated with stabilization of endogenous FVIII may add to the risk. It is advised that clinicians monitor FVIII daily and avoid high levels. Venous thromboprophylaxis should be considered [11].

Monitoring therapy

A survey of experienced clinicians in Europe reported that for surgery 22 of 24 responders measured FVIII daily and 18 of

24 measured VWF:RCo daily. Only 5 of 24 monitored the bleeding time and two of these only for the first postoperative day [4]. A survey of 194 US clinicians reported that for major surgery the targeted levels for both FVIII and VWF:RCo were greater than 80%; targeted levels greater than 50% were considered adequate for minor surgery [54].

Factor VIII

It is standard practice to monitor levels of FVIII. Monitoring of FVIII alone, however, does not constitute adequate hemostatic assessment. Measurement of VWF:RCo, particularly in patients with type 2 and 3 VWD, is also needed. In these situations the VWF:RCo may be considerably lower than the FVIII, creating a false sense of clinical security.

VWF:RCo

It has been recommended that VWF:RCo should be monitored, targeting levels appropriate for the clinical situation (Table 48.4) [5]. This represents reasonable clinical practice [4].

Bleeding time

A retrospective study of 76 patients unresponsive to DDAVP undergoing surgical procedures showed no correlation between correction of the bleeding time and surgical hemostasis. Furthermore, clinicians did not alter their management dependent on the bleeding time [61]. Adequate hemostasis has been achieved with concentrates that do not reproducibly correct the bleeding time [26]. The bleeding time may be useful if patients are bleeding abnormally despite replacement therapy. Some patients whose bleeding time is not corrected with infused VWF may respond to platelet infusions [62].

PFA-100

Platelet function analyzer (PFA-100) has been used to monitor replacement therapy in patients with VWD. Although convenient to use, the reliability of this method of monitoring hemostasis following the use of VWF-containing concentrates is not yet established and results should be interpreted with caution.

Treatment failure

Patients who have an inadequate clinical response to VWF-containing concentrates despite correction of laboratory parameters may respond to infusion of platelets or cryoprecipitate. Surgical bleeding, inhibitory activity, and other causes of hemostatic defects should be considered.

Platelets

The bleeding time may be shortened by platelet infusion [62]. If mucosal bleeding persists and the bleeding time remains prolonged after adequate replacement therapy with a VWF-containing concentrate, platelet infusions should be considered.

Cryoprecipitate

Cryoprecipitate is not virally inactivated and should not be used for the management of VWD unless other treatment modalities have failed or are unavailable [4,5]. Some patients who have not responded to a VWF-containing concentrate may respond to cryoprecipitate [5].

Conclusion

von Willebrand factor-containing concentrates play a valuable role in the management of patients with VWD who are not candidates for treatment with DDAVP. Further, clinical trials that focus on hemostatic endpoints are required to investigate how to improve the use and monitoring of these products. The potential use of recombinant VWF concentrate is under investigation.

References

1 Mannucci PM. Desmopressin (DDAVP) in the treatment of bleeding disorders: the first 20 years. *Blood* 1997; **90**: 2515–21, 1–10.
2 Makris M, Baglin T, Dusheiko G, *et al.*, and Transfusion Transmitted Infection Working Party of the UK Haemophilia Centre Directors Organization. Guidelines on the diagnosis, management and prevention of hepatitis in haemophilia. *Haemophilia* 2001; **7**: 339–45.
3 Federici AB, Castaman G, Mannucci PM, and Italian Association of Hemophilia Centers (AICE). Guidelines for the diagnosis and management of von Willebrand disease in Italy. *Haemophilia* 2002; **8**: 607–21.
4 Lusher JM. Clinical guidelines for treating von Willebrand disease patients who are not candidates for DDAVP—a survey of European physicians. *Haemophilia* 1998; **4**(Suppl. 3): 11–14.
5 Pasi KJ, Collins PW, Keeling DM, *et al.* Management of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organization. *Haemophilia* 2004; **10**: 218–31.
6 Smith TJ, Gill JC, Ambruso DR, Hathaway WE. Hyponatremia and seizures in young children given DDAVP. *Am J Hematol* 1989; **31**: 199–202.
7 Bond L, Bevan D. Myocardial infarction in a patient with hemophilia treated with DDAVP. *N Engl J Med* 1988; **318**: 121.
8 Grunwald Z, Sather SD. Intraoperative cerebral infarction after desmopressin administration in infant with end-stage renal disease. *Lancet* 1995; **345**: 1364–5.

9 Byrnes JJ, Larcada A, Moake JL. Thrombosis following desmopressin for uremic bleeding. *Am J Hematol* 1988; **28**: 63–5.

10 Makris M, Colvin B, Gupta V, Shields ML, Smith MP. Venous thrombosis following the use of intermediate purity FVIII concentrate to treat patients with von Willebrand's disease. *Thromb Haemost* 2002; **88**: 387–8.

11 Mannucci PM. Venous thromboembolism in von Willebrand disease. *Thromb Haemost* 2002; **88**: 378–9.

12 Holmberg L, Nilsson IM, Borge L, Gunnarsson M, Sjorin, E. Platelet aggregation induced by 1-desamino-8-D-arginine vasopressin (DDAVP) in Type IIB von Willebrand's disease. *N Engl J Med* 1983; **309**: 816–21.

13 Casonato A, Pontara E, Dannhaeuser D, *et al.* Re-evaluation of the therapeutic efficacy of DDAVP in type IIB von Willebrand's disease. *Blood Coagul Fibrinolysis* 1994; **5**: 959–64.

14 Fowler WE, Berkowitz LR, Roberts HR. DDAVP for type IIB von Willebrand disease. *Blood* 1989; **74**: 1859–60.

15 Gralnick HR, Williams SB, McKeown LP, *et al.* DDAVP in type IIa von Willebrand's disease. *Blood* 1986; **67**: 465–8.

16 Michiels JJ, van de V, van Vliet HH, van der Planken M, Schroyens W, Berneman Z. Response of von Willebrand factor parameters to desmopressin in patients with type 1 and type 2 congenital von Willebrand disease: diagnostic and therapeutic implications. *Semin Thromb Hemost* 2002; **28**: 111–32.

17 Mazurier C, Gaucher C, Jorieux S, Goudemand M. Biological effect of desmopressin in eight patients with type 2N ('Normandy') von Willebrand disease. Collaborative Group. *Br J Haematol* 1994; **88**: 849–54.

18 Goudemand J, Negrier C, Ounnoughene N, Sultan Y. Clinical management of patients with von Willebrand's disease with a VHP vWF concentrate: the French experience. *Haemophilia* 1998; **4**(Suppl. 3): 48–52.

19 Castaman G, Lethagen S, Federici AB, *et al.* Response to desmopressin is influenced by the genotype and phenotype in type 1 von Willebrand disease (VWD): results from the European Study MCMDM-1VWD. *Blood* 2008; **111**: 3531–9.

20 Mannucci PM, Lombardi R, Bader R, *et al.* Heterogeneity of type I von Willebrand disease: evidence for a subgroup with an abnormal von Willebrand factor. *Blood* 1985; **66**: 796–802.

21 Casonato A, Pontara E, Sartorello F, *et al.* Reduced von Willebrand factor survival in type Vicenza von Willebrand disease. *Blood* 2002; **99**: 180–4.

22 Haberichter SL, Castaman G, Budde U, *et al.* Identification of type 1 von Willebrand disease patients with reduced von Willebrand factor survival by assay of the VWF propeptide in the European study: molecular and clinical markers for the diagnosis and management of type 1 VWD (MCMDM-1VWD). *Blood* 2008; **111**: 4979–85.

23 Menache D, Aronson DL. New treatments of von Willebrand disease: plasma derived von Willebrand factor concentrates. *Thromb Haemost* 1997; **78**: 566–70.

24 Hubbard AR. von Willebrand factor standards for plasma and concentrate testing. *Semin Thromb Hemost* 2006; **32**: 522–8.

25 Mannucci PM, Tenconi PM, Castaman G, Rodeghiero F. Comparison of four virus-inactivated plasma concentrates for treatment of severe von Willebrand disease: a cross-over randomised trial. *Blood* 1992; **79**: 3130–7.

26 Mannucci PM, Moia M, Rebulla P, Altieri D, Monteagudo J, Castillo R. Correction of the bleeding time in treated patients with severe von Willebrand disease is not solely dependent on the normal multimeric structure of plasma von Willebrand factor. *Am J Hematol* 1987; **25**: 55–65.

27 Auerswald G, Eberspacher B, Engl W, *et al.* Successful treatment of patients with von Willebrand disease using a high-purity double-virus inactivated factor VIII/von Willebrand factor concentrate (Immunate). *Semin Thromb Hemost* 2002; **28**: 203–14.

28 Bello IF, Yuste VJ, Molina MQ, Navarro FH. Fanhdi, efficacy and safety in von Willebrand's disease: prospective international study results. *Haemophilia* 2007; **13**(Suppl. 5): 25–32.

29 Berntorp E, Nilsson IM. Use of a high-purity factor VIII concentrate (Hemate P) in von Willebrand's disease. *Vox Sanguinis* 1989; **56**: 212–17.

30 Berntorp E, Archey W, Auerswald G, *et al.* A systematic overview of the first pasteurised VWF/FVIII medicinal product, Haemate P/Humate-P: history and clinical performance. *Eur J Haematol* 2008 (Suppl.): 3–35.

31 Chtourou S, Porte P, Nogre M, *et al.* A solvent/detergent-treated and 15-nm filtered factor VIII: a new safety standard for plasma-derived coagulation factor concentrates. *Vox Sanguinis* 2007; **92**: 327–37.

32 Cumming AM, Fildes S, Cumming IR, Wensley RT, Redding OM, Burn AM. Clinical and laboratory evaluation of National Health Service factor VIII concentrate (8Y) for the treatment of von Willebrand's disease. *Br J Haematol* 1990; **75**: 234–9.

33 Federici AB, Baudo F, Caracciolo C, *et al.* Clinical efficacy of highly purified, doubly virus-inactivated factor VIII/von Willebrand factor concentrate (Fanhdi) in the treatment of von Willebrand disease: a retrospective clinical study. *Haemophilia* 2002; **8**: 761–7.

34 Federici AB. Highly purified VWF/FVIII concentrates in the treatment and prophylaxis of von Willebrand disease: the PRO.WILL Study. *Haemophilia* 2007; **13**(Suppl. 5): 15–24.

35 Franchini M, Rossetti G, Tagliaferri A, *et al.* Efficacy and safety of factor VIII/von Willebrand's factor concentrate (Haemate-P) in preventing bleeding during surgery or invasive procedures in patients with von Willebrand disease. *Haematologica* 2003; **88**: 1279–83.

36 Gill JC, Ewenstein BM, Thompson AR, Mueller-Velten G, Schwartz BA, Humate P. Successful treatment of urgent bleeding in von Willebrand disease with factor VIII/VWF concentrate (Humate-P): use of the ristocetin cofactor assay (VWF:RCo) to measure potency and to guide therapy. *Haemophilia* 2003; **9**: 688–95.

37 Goudemand J, Mazurier C, Marey A, *et al.* Clinical and biological evaluation in von Willebrand's disease of a von Willebrand factor concentrate with low factor VIII activity. *Br J Haematol* 1992; **80**: 214–21.

38 Lubetsky A, Schulman S, Varon D, *et al.* Safety and efficacy of continuous infusion of a combined factor VIII-von Willebrand factor (vWF) concentrate (Haemate-P) in patients with von Willebrand disease. *Thromb Haemost* 1999; **81**: 229–33.

39 Mannucci PM, Chediak J, Hanna W, *et al.*, and Alphanate Study Group. Treatment of von Willebrand disease with a high-purity factor VIII/von Willebrand factor concentrate: a prospective, multicenter study. *Blood* 2002; **99**: 450–6.

40 Pasi KJ, Williams MD, Enayat MS, Hill FG. Clinical and laboratory evaluation of the treatment of von Willebrand's disease patients with heat-treated factor VIII concentrate (BPL 8Y). *Br J Haematol* 1990; **75**: 228–33.

41 Rivard GE, Aledort L. Efficacy of factor VIII/von Willebrand factor concentrate Alphanate in preventing excessive bleeding during surgery in subjects with von Willebrand disease. *Haemophilia* 2008; **14**: 271–5.

42 Shortt J, Dunkley S, Rickard K, Baker R, Street A. Efficacy and safety of a high purity, double virus inactivated factor VIII/von

Willebrand factor concentrate (Biostate) in patients with von Willebrand disorder requiring invasive or surgical procedures. *Haemophilia* 2007; **13**: 144–8.

43 Mazurier C. Composition, quality control, and labeling of plasma-derived products for the treatment of von Willebrand disease. *Semin Thromb Hemost* 2006; **32**: 529–36.

44 Kreuz W, Mentzer D, Becker S, Scharrer I, Kornhuber B. Haemate P in children with von Willebrand's disease. *Haemostasis* 1994; **24**: 304–10.

45 Lethagen S, Kyrle PA, Castaman G, Haertel S, Mannucci PM, and Haemate P Surgical Study Group. von Willebrand factor/factor VIII concentrate (Haemate P) dosing based on pharmacokinetics: a prospective multicenter trial in elective surgery. *J Thromb Haemost* 2007; **5**: 1420–30.

46 Mazurier C, De Romeuf C, Parquet-Gernez A, Goudemand M. *In vitro* and *in vivo* characterization of a high-purity, solvent/detergent-treated factor VIII concentrate: evidence for its therapeutic efficacy in von Willebrand's disease. *Eur J Haematol* 1989; **43**: 7–14.

47 Thompson AR, Gill JC, Ewenstein BM, Mueller-Velten G, Schwartz BA, Humate, P. Successful treatment for patients with von Willebrand disease undergoing urgent surgery using factor VIII/VWF concentrate (Humate-P). *Haemophilia* 2004; **10**: 42–51.

48 Borel-Derlon A, Federici A, Roussel-Robert V, *et al.* Treatment of severe von Willebrand disease with a high-purity von Willebrand factor concentrate (Wilfactin): a prospective study of 50 patients. *J Thromb Haemost* 2007; **5**: 1115–1124.

49 Dobrkovska A, Krzensk U, Chediak JR. Pharmacokinetics, efficacy and safety of Humate-P in von Willebrand disease. *Haemophilia* 1998; **4**(Suppl. 3): 33–9.

50 Favaloro EJ, Lloyd J, Rowell J, *et al.* Comparison of the pharmacokinetics of two von Willebrand factor concentrates [Biostate and AHF (High Purity)] in people with von Willebrand disorder. A randomised cross-over, multi-centre study. *Thromb Haemost* 2007; **97**: 922–30.

51 Goudemand J, Scharrer I, Berntorp E, *et al.* Pharmacokinetic studies on Wilfactin, a von Willebrand factor concentrate with a low factor VIII content treated with three virus-inactivation/removal methods. *J Thromb Haemost* 2005; **3**: 2219–27.

52 Lubetsky A, Martinowitz U, Luboshitz J, *et al.* Efficacy and safety of a factor VIII-von Willebrand factor concentrate 8Y: stability, bacteriological safety, pharmacokinetic analysis and clinical experience. *Haemophilia* 2002; **8**: 622–8.

53 Lillicrap D, Poon MC, Walker I, Xie F, Schwartz BA, and Association of Hemophilia Clinic Directors of Canada. Efficacy and safety of the factor VIII/von Willebrand factor concentrate, haemate-P/humate-P: ristocetin cofactor unit dosing in patients with von Willebrand disease. *Thromb Haemost* 2002; **87**: 224–30.

54 Cohen AJ, Kessler CM, Ewenstein BM, and Hemophilia Research Society of North America. Management of von Willebrand disease: a survey on current clinical practice from the haemophilia centres of North America. *Haemophilia* 2001; **7**: 235–41.

55 Berntorp E, Petrini P. Long-term prophylaxis in von Willebrand disease. *Blood Coagul Fibrinolysis* 2005; **16**(Suppl. 1): S23–6.

56 Berntorp E, Abshire T, and von Willebrand Disease Prophylaxis Network Steering Committee. The von Willebrand disease prophylaxis network: exploring a treatment concept. *J Thromb Haemost* 2006; **4**: 2511–12.

57 Mannucci PM, Tamaro G, Narchi G, *et al.* Life-threatening reaction to factor VIII concentrate in a patient with severe von Willebrand disease and alloantibodies to von Willebrand factor. *Eur J Haematol* 1987; **39**: 467–70.

58 Ciavarella N, Schiavoni M, Valenzano E, Mangini F, Inchingolo F. Use of recombinant factor VIIa (NovoSeven) in the treatment of two patients with type III von Willebrand's disease and an inhibitor against von Willebrand factor. *Haemostasis* 1996; **26**(Suppl. 1): 150–4.

59 Kumar S, Pruthi RK, Nichols WL. Acquired von Willebrand's syndrome: a single institution experience. *Am J Hematol* 2003; **72**: 243–7.

60 Smith MP, Rice KM, Bromidge ES, *et al.* Continuous infusion therapy with very high purity von Willebrand factor concentrate in patients with severe von Willebrand disease. *Blood Coagul Fibrinolysis* 1997; **8**: 6–12.

61 Foster PA. A perspective on the use of FVIII concentrates and cryoprecipitate prophylactically in surgery or therapeutically in severe bleeds in patients with von Willebrand disease unresponsive to DDAVP: results of an international survey. On behalf of the Subcommittee on von Willebrand Factor of the Scientific and Standardization Committee of the ISTH. *Thromb Haemost* 1995; **74**: 1370–8.

62 Castillo R, Monteagudo J, Escolar G, Ordinas A, Magallon M, Villar JM. Hemostatic effect of normal platelet transfusion in severe von Willebrand disease patients. *Blood* 1991; **77**: 1901–5.

49 Factor II

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Factor II (prothrombin) deficiency is a rare bleeding disorder that in the homozygous severe form is associated with high mortality. Milder deficiencies, however, may actually be underdiagnosed, since routine laboratory screening tests are often only slightly affected and many patients do not experience bleeding symptoms requiring medical care. This chapter will provide an overview of the structure and function of prothrombin and some clinical perspectives on the various deficiency states.

Biosynthesis

Prothrombin is synthesized as a preproprotein in hepatocytes and encoded by a gene, on chromosome 11, of 21 kb containing 14 exons and 13 intervening sequences [1]. The prepeptide directs the synthesized protein to the endoplasmic reticulum and is then removed prior to the process of post-translational modification. The mature molecule is a plasma glycoprotein and zymogen of a serine protease requiring vitamin K for normal biosynthesis [2]. The common feature of all vitamin K-dependent proteases is an N-terminal noncatalytic module containing γ-carboxyglutamic acid (Gla) residues, but, unlike the procoagulant factor VII (FVII), FIX, and FX, prothrombin consists not of epidermal growth factor (EGF)-like modules but of two kringle domains separated from the Gla module by a disulfide loop. The propeptide serves as an anchor for the γ-carboxylase, and is cleaved off before secretion [3]. The catalytic serine protease part contains the active site and is located at the C-terminal end of the molecule.

Structure and function

The binding of calcium to the Gla module is fundamental for proper folding of the vitamin K-dependent enzymes and for the accumulation of the factors on a negatively charged phospholipid membrane at concentrations high enough to promote fibrin formation [4,5]. The Gla residues have also been associated with normal intracellular transportation. The function of the kringles, named after a pastry because of their pretzel-like form, is to some extent unclear, although the second kringle seems to be involved in the binding of FVa and thrombin. Thrombin is the active form of prothrombin. It is formed by the cleavage of two peptide bonds by the prothrombinase complex composed of FXa, FVa, and calcium on a phospholipid surface (Figure 49.1). In contrast to the other activated vitamin K-dependent coagulation factors, thrombin contains none of the noncatalytic modules and therefore has no phospholipid-binding capacity, but dissociates from the prothrombinase complex by diffusion. Thrombin exerts several pro- and anticoagulant effects. It triggers platelet aggregation and promotes coagulation by activating regulatory pathways and generating fibrin monomers by cleavage of a peptide bond in each of the α- and β-subunits of fibrinogen [6].

Prothrombin deficiency

Congenital prothrombin deficiency was first described by Quick in 1947 and further explored in subsequent reports [7,8]. The condition is inherited as an autosomal recessive trait and has been extensively reviewed by Girolami and coworkers [9]. The prevalence of congenital prothrombin deficiency is low, reported in the range of 1 : 1 000 000–2 000 000 [10,11]. Based on the immunoreactive component in plasma and the functional activity, two different phenotypic deficiencies have been described. In type 1 deficiency, or hypoprothrombinemia, the levels of antigen and functional activity are decreased to a similar extent, whereas in type 2 deficiency, or dysprothrombinemia, the enzyme itself is synthesized and present in plasma at a more or less normal level, but the coagulant activity is low. From what is presently known, complete prothrombin deficiency appears to be incompatible with life [12]. Mutations found in patients with hypo- and dysprothrombinemia have been reviewed and are summarized in Figure 49.2 [9,11,13–18]. The mutations associated with dysprothrombinemia are missense mutations, most of which interfere with the FXa binding site and the active site in the serine protease domain. In patients with hypoprothrombinemia, nonsense and small deletions have also been found, but the mechanisms by which these defects affect function largely remain to be settled. Compound heterozygosity for dys- and hypoprothrombinemia has been described [19]. In addition to isolated prothrombin deficiency, combined defects with the other vitamin K-dependent coagulation factors VII, IX, X, and protein C and S have been found with variable clinical manifestations and in some cases associated with skeletal

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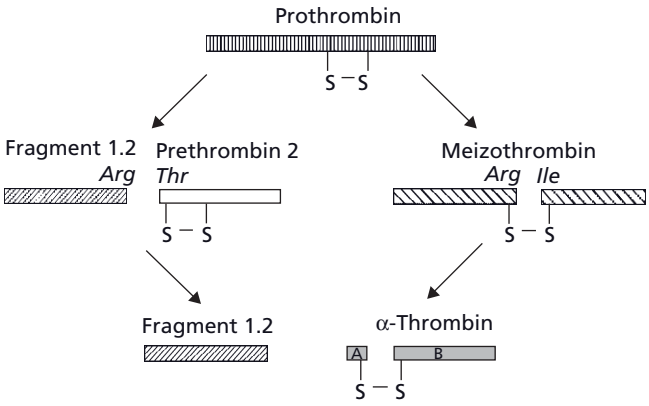

Figure 49.1 Schematic overview of the pathways for prothrombin activation. a-Thrombin is formed by the cleavage of two peptide bonds in prothrombin (Arg271–|–Thr272) and (Arg320–|–Ile321). Depending on which bond is cleaved first, fragment 1.2 and prethrombin 2 or meizothrombin is formed as an intermediate product. The activation by the prothrombinase complex is thought to mainly proceed through the formation of meizothrombin.

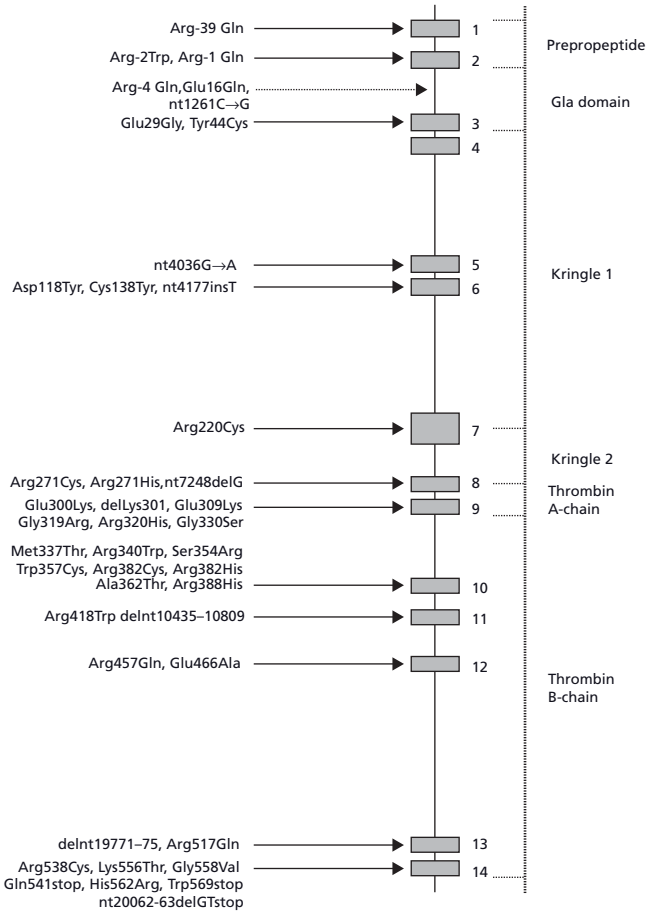

Figure 49.2 Mutations in the prothrombin gene associated with prothrombin deficiency projected on the corresponding exons and protein domains. Modified from [11].

abnormalities [20–25]. The molecular defects are thought to reside in the gene of the γ-glutamyl carboxylase [26,27].

Acquired prothrombin deficiency is usually associated with neutralizing anticoagulants, the lupus anticoagulant-hypoprothrombinemia syndrome (LA-HPS), in patients with systemic lupus erythematosus (SLE), or following viral infections [28–31]. However, non-neutralizing antibodies accelerating the clearance of prothrombin have also been described [32,33].

Laboratory diagnosis

Specific tests are usually required to identify prothrombin deficiency. Screening tests such as prothrombin time (PT) and activated partial thromboplastin time (aPTT) are variably prolonged and in milder cases often more or less normal, suggesting that milder cases could be underdiagnosed [34]. Several specific diagnostic tools are available, some of which are based on viper venoms [9]. The most widely used test is a one-stage assay using thromboplastin as the activating agent. The test is reliable for screening purposes. In patients with homozygous type 1 prothrombin deficiency activity is usually less than 10% of normal, whereas subjects heterozygous for the same deficiency usually have values between 40% and 60%. Type 1 deficient patients generally have similar results regardless of the diagnostic method used, whereas the results for patients with type 2 deficiency may be inconsistent from method to method. Immunologic methods are required to fully characterize the deficient state in terms of true hypo- or dysprothrombinemia.

Clinical manifestations

Clinically, heterozygotic patients with prothrombin deficiency are usually asymptomatic, but homozygote subjects with activity levels less than 10% of normal may experience several different bleeding symptoms [9,35]. In general, patients with dysprothrombinemia seem to have more variable and milder symptoms than patients with hypoprothrombinemia. In a cohort of 14 patients with a plasma level of 4–10%, mucocutaneous bleeds from the nose, gums, and uterus were the most frequent bleeding symptoms. Joint and muscle bleeds were also relatively frequent and found in approximately one-third of the patients, some of whom developed arthropathy. Morbidity and mortality owing to intracranial hemorrhage are high in patients with severe deficiency. In women, the risk of ovulation-induced hemoperitoneum should be considered [36].

Therapeutic aspects

Prothrombin levels of 25–30 IU/dL are required for normal hemostasis, but in the case of life-threatening bleeds and major

surgery higher levels may be needed [37]. Consequently, in many patients with a coagulant activity above 30% only antifibrinolytics will be required. In patients with more severe deficiency, however, replacement with the deficient factor must be given for surgery and in cases of trauma and more serious bleeding episodes. No pure prothrombin concentrate is available, but treatment can be given with either plasma and/or prothrombin complex concentrates. The amount of plasma needed for transfusion is usually about 15–20 mL/kg body weight (b.w.), whereas prothrombin complex concentrates containing around 1 IU of prothrombin per unit of FIX can be given in a dose of 20–30 IU/kg. Taking the long half-life (72 h) of prothrombin into account, the infusion intervals must be adjusted to the clinical situation in order not to accumulate the enzyme and increase the risk of thrombotic events. In the current era of manufacturing processes, the risk of transmitting viruses is considered very low, although not negligible. Patients with acquired hypoprothrombinemia often require immunomodulating agents and usually respond to corticosteroids, cyclophosphamide, danazol, and/or intravenous gammaglobulins [38–42].

Conclusion

Prothrombin deficiency is rare, but it is important to recognize as patients with severe deficiency may experience life-threatening bleeding symptoms. Patients with milder deficiencies are probably underdiagnosed. An improved awareness of this condition may not only improve patient care, but also contribute to a better understanding of the hemostatic process.

References

1 Degen SJ, Davie EW. Nucleotide sequence of the gene for human prothrombin. *Biochemistry* 1987; **26**: 6165–77.
2 Furie B, Furie BC. The molecular basis of blood coagulation. *Cell* 1988; **53**: 505–18.
3 Furie B, Furie BC. Molecular basis of gamma-carboxylation. Role of the propeptide in the vitamin K-dependent proteins. *Ann NY Acad Sci* 1991; **614**: 1–10.
4 Swanson JC, Suttie JW. Prothrombin biosynthesis: characterization of processing events in rat liver microsomes. *Biochemistry* 1985; **24**: 3890–7.
5 Zhang L, Castellino FJ. Influence of specific gamma-carboxyglutamic acid residues on the integrity of the calcium-dependent conformation of human protein C. *J Biol Chem* 1992; **267**: 26078–84.
6 Hemker HC. Thrombin generation, an essential step in hemostasis and thrombosis. In: Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD (eds.) *Hemostasis and Thrombosis*, 3rd edn. Edinburgh: Churchill Livingstone, 1994: 477–90.
7 Quick AJ. Congenital hypoprothrombinemia and pseudo-hypoprothrombinemia. *Lancet* 1947; **ii**: 379–82.
8 Quick AJ, Hussey CV. Hereditary hypoprothrombinemias. *Lancet* 1962; **i**: 173–7.
9 Girolami A, Scarano L, Saggiorato G, *et al.* Congenital deficiencies and abnormalities of prothrombin. *Blood Coagul Fibrinol* 1998; **9**: 557–69.
10 Tuddenham EGD, Cooper DN. *The Molecular Genetics of Hemostasis and its Inherited Disorders*. Oxford: Oxford University Press, 1994.
11 Peyvandi F, Duga S, Akhavan S, Mannucci PM. Rare coagulation deficiencies. *Haemophilia* 2002; **8**: 308–21.
12 Sun WY, Witte DP, Degen JL, *et al.* Prothrombin deficiency results in embryonic and neonatal lethality in mice. *Proc Natl Acad Sci USA* 1998; **95**: 7597–602.
13 Akhavan S, Mannucci PM, Lak M, *et al.* Identification and three-dimensional structural analysis of nine novel mutations in patients with prothrombin deficiency. *Thromb Haemost* 2000; **84**: 989–97.
14 Girolami A, Santarossa L, Scarparo P, Candeo N, Girolami B. True congenital prothrombin deficiency due to a "new" mutation in the prepropeptide (Arg-39 GLN). *Acta Haematol* 2008; **120**: 82–6.
15 Wang W, Fu Q, Zhou R, *et al.* Prothrombin Shanghai: hypoprothrombinaemia caused by substitution of Gla29 by Gly. *Haemophilia* 2004; **10**: 94–7.
16 Jayandharan G, Wiswabandya A, Baidya S, *et al.* Molecular genetics of hereditary prothrombin deficiency in Indian patients: identification of a novel Ala362→Thr (Prothrombin Vellore 1) mutation. *J Thromb Haemost* 2005; **3**: 1446–53.
17 Lefkowitz JB, Weller A, Nuss R, Santiago-Borrero PJ, Brown DL, Ortiz IR. A common mutation, Arg457→Gln, links prothrombin deficiencies in the Puerto Rican population. *J Thromb Haemost* 2003; **1**: 2381–8.
18 Wong AYK, Hewitt J, Clarke BJ, *et al.* Severe prothrombin deficiency caused by prothrombin-Edmonton (R-4Q) combined with a previously undetected deletion. *J Thromb Haemost* 2006; **4**: 2623–8.
19 Akhavan S, Luciani M, Lavoretano S, Mannucci PM. Phenotypic and genetic analysis of a compound heterozygote for dys- and hypoprothrombinaemia. *Br J Haematol* 2003; **120**: 142–4.
20 Goldsmith GH, Jr., Pence RE, Ratnoff OD, *et al.* Studies on a family with combined functional deficiencies of vitamin K-dependent coagulation factors. *J Clin Invest* 1982; **69**: 1253–60.
21 Vicente V, Maia R, Alberca I, *et al.* Congenital deficiency of vitamin K-dependent coagulation factors and protein C. *Thromb Haemost* 1984; **51**: 343–6.
22 Pauli RM, Lian JB, Mosher DF, Suttie JW. Association of congenital deficiency of multiple vitamin K-dependent coagulation factors and the phenotype of the warfarin embryopathy: clues to the mechanism of teratogenicity of coumarin derivatives. *Am J Hum Genet* 1987; **41**: 566–83.
23 Brenner B, Tavori S, Zivelin A, *et al.* Hereditary deficiency of all vitamin K-dependent procoagulants and anticoagulants. *Br J Haematol* 1990; **75**: 537–42.
24 Boneh A, Bar-Ziv J. Hereditary deficiency of vitamin K-dependent coagulation factors with skeletal abnormalities. *Am J Med Genet* 1996; **65**: 241–3.
25 McMahon MJ, James AH. Combined deficiency of factors II, VII, IX, and X (Borgschulte-Grigsby deficiency) in pregnancy. *Obstet Gynecol* 2001; **97**: 808–9.
26 Mutucumarana VP, Stafford DW, Stanley TB, *et al.* Expression and characterization of the naturally occurring mutation L394R in human ?-glutamyl carboxylase. *J Biol Chem* 2000; **275**: 32572–7.

27 Brenner B. Hereditary deficiency of vitamin K-dependent coagulation factors. *Thromb Haemost* 2000; **84**: 935–6.
28 Bernini JC, Buchanan GR, Ashcraft J. Hypoprothrombinemia and severe hemorrhage associated with a lupus anticoagulant. *J Pediatr* 1993; **123**: 937–9.
29 Galli M, Barbui T. Antiprothrombin antibodies: detection and clinical significance in the antiphospholipid syndrome. *Blood* 1999; **93**: 2149–57.
30 Schmugge M, Tolle S, Marbet GA, *et al.* Gingival bleeding, epistaxis and haematoma three days after gastroenteritis: the haemorrhagic lupus anticoagulant syndrome. *Eur J Pediatr* 2001; **160**: 43–6.
31 Baca V, Montiel G, Meillon L, *et al.* Diagnosis of lupus anticoagulant in the lupus anticoagulant-hypoprothrombinemia syndrome: report of two cases and review of the literature. *Am J Hematol* 2002; **71**: 200–7.
32 Bajaj SP, Rapaport SI, Barclay S, Herbst KD. Acquired hypoprothrombinemia due to non-neutralizing antibodies to prothrombin: mechanism and management. *Blood* 1985; **65**: 1538–43.
33 Lee ES, Hibsman BK, Liebman HA. Acquired bleeding disorder in a patient with malignant lymphoma: antibody-mediated prothrombin deficiency. *Cancer* 2001; **91**: 636–41.
34 Girolami A, De Marco L, Dal Bo Zanon R, *et al.* Rare quantitative and qualitative abnormalities of coagulation. *Clin Haematol* 1985; **14**: 385–441.
35 Peyvandi F, Mannucci PM. Rare coagulation disorders. *Thromb Haemost* 1999; **82**: 1207–14.
36 Girolami A, Lombardi AM, Candeo N, Scarparo P, Paternoster A. Control of ovulation-induced hemoperitoneum by oral contraceptives in a patient with congenital hypoprothrombinemia and in another with congenital factor V deficiency. *Acta Haematol* 2008; **119**: 236–40.
37 Mannucci PM, Duga S, Peyvandi F. Recessively inherited coagulation disorders. *Blood* 2004; **104**: 1243–52.
38 Simel DL, St Clair EW, Adams J, Greenberg CS. Correction of hypoprothrombinemia by immunosuppressive treatment of the lupus anticoagulant-hypoprothrombinemia syndrome. *Am J Med* 1987; **83**: 563–6.
39 Hift RJ, Bird AR, Sarembock BD. Acquired hypoprothrombinaemia and lupus anticoagulant: response to steroid therapy. *Br J Rheumatol* 1991; **30**: 308–10.
40 Williams S, Linardic C, Wilson O, *et al.* Acquired hypoprothrombinemia: effects of danazol treatment. *Am J Hematol* 1996; **53**: 272–6.
41 Pernod G, Arvieux J, Carpentier PH, *et al.* B. Successful treatment of lupus anticoagulant-hypoprothrombinemia syndrome using intravenous immunoglobulins. *Thromb Haemost* 1997; **78**: 969–70.
42 Wong RS, Lau FY, Cheng G. Successful treatment of acquired hypoprothrombinemia without associated lupus anticoagulant using intravenous immunoglobulin. *Haematologica* 2001; **86**: 551.

50 Factor V and combined factor V and VIII deficiencies

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Factor V deficiency

Within the coagulation cascade, factor V (FV) is one essential nonenzymatic cofactor of the prothrombinase complex, which catalyses the conversion of prothrombin into thrombin [1]. This enzyme complex consists of activated FV (FVa), calcium, phospholipids, and activated factor X (FXa). FVa increases the concentration of FXa at the membrane surface by acting as a receptor for FXa and allosterically alters the active site of FXa to optimize its ability to cleave prothrombin. By stabilizing the complex and increasing the rate at which FXa cleaves prothrombin, FVa enhances prothrombin activation by five orders of magnitude when compared with FXa alone.

Although most FV is present in plasma, approximately 20–25% of the circulating FV is found within platelet α-granules. The source of platelet FV has not been definitively established, but evidence indicates that platelets or megakaryocytes can both endocytose and synthesize FV. Platelet FV is partially proteolysed and is stored bound to the protein multimerin in α-granules [2,3].

Kingsley first described the autosomal recessive inheritance pattern of congenital FV deficiency (Online Mendelian Inheritance in Man, OMIM +227400) in two South African families of Dutch ancestry [4]. However, only in 1987 was the cDNA cloned and the amino acid sequence of the protein determined [5]. The entire genomic structure of the *F5* gene was characterized in 1992 [6].

Aside from mutations in the *F5* gene, a reduction of the circulating level of FV can also been observed in combined FV and FVIII deficiency (described later). Deficiency of FV can also arise because of acquired inhibitors to FV and defects that affect the storage and processing of FV [7,8].

No precise epidemiologic data exist for congenital FV deficiency, but its prevalence has been estimated to be 1 in 1 000 000 persons [9]. Unfortunately, the worldwide prevalence of FV deficiency compared with other rare bleeding disorders (RBDs) is not available, because a reliable picture of the global distribution of these disorders could not be developed. This is because of the limited number of reliable national registries, especially in developing countries, that make available data to be not homogeneous. However, an indication of the worldwide prevalence of FV deficiency can be derived from the World Federation of Hemophilia (WFH) global survey (WFH 2007, courtesy of Dr. Mark Brooker) and the RBD database (RBDD, www.rbdd.org) reporting that patients affected by FV deficiency seem to represent ~11% of the total number of patients affected by RBDs.

Factor V protein and gene structure

Human FV is synthesized by hepatocytes and megakaryocytes. Approximately 75% of FV is secreted, circulating in blood as a precursor molecule, whereas the remaining 25% is stored into the platelet α-granules [10]. FV shows high functional and structural homology with factor VIII (FVIII) [11]. Both these proteins have the same A1–A2–B–A3–C1–C2 structure: the three A domains of FV and FVIII share approximately 30% amino acid identity with each other and with the triplicated A domain of ceruloplasmin, the major plasma copper-transport protein [12]. The large B domain, having no homology with other proteins, is proteolitically removed during activation of FV and FVIII. Both C domains are tandem modules of approximately 150 amino acids and they are part of the major subfamily of discodein domains [13].

So far, only the three-dimensional structure of the C2 domain of human FV has been determined by X-ray crystallography; the C2 domain shows an eight-stranded β-barrel core from which three spikes protrude. These spikes mediate binding of FVa to phospholipid membranes [14]. The description of the crystal structure of activated protein C–inactivated bovine FVa allowed the reconstruction of a complete molecular model for FVa [15], showing that the two C domains are aligned "edge to edge" to form a platform that lifts the A domains to an appropriate height above the membrane for interaction with their physiologic partners. Very recently, this

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model was substantially confirmed by the low-resolution human FVa three-dimensional structure, obtained by Stoilova-McPhie and colleagues [16], and the solvent-equilibrated model of human FVa proposed by Lee and coworkers [17], which also indicated a possible significant shift toward planarity in the arrangement of the five FVa domains.

The FV which circulates in the blood is a single glycosylated polypeptide of 330 kDa that, through proteolytic cleavages at three arginine residues (Arg709, Arg1018, and Arg1545) [18], releases the B domain and creates a dimeric molecule composed of a 105-kDa heavy chain that contains the A1 and A2 domains and a 71- to 74-kDa light chain that contains the A3, C1, and C2 domains. These two chains are held together by calcium and hydrophobic interactions. The heavy chain provides the contacts for both FXa and prothrombin, whereas the two C domains in the light chain are needed for the interaction of FVa with the phospholipid surface. The A3 domain in the light chain is involved in both FXa and phospholipid interactions. The two FVa chains link FXa to the phospholipid's surface formed by the platelet plug at the site of injury and enable FXa to efficiently bind and cleave prothrombin to generate thrombin [19].

Cleavage by activated protein C (aPC) at three arginine residues (Arg306, Arg506, and Arg679) located in the FV heavy chain culminates in the inactivation of the procoagulant activity [20]. The cleavage at Arg506 reduces both the cofactor activity and its affinity for FXa, and the cleavage at Arg306 completes the inactivation. Once cleaved at Arg506, FVa is converted to FVac (FV anticoagulant), which interacts with aPC and protein S to inactivate FVIIIa. Thus, aPC not only turns off the FVa procoagulant activity but also converts it to an anticoagulant [19].

FV is indispensable for life, as was demonstrated by experimental knockout mice lacking the FV gene, which die either *in utero* at embryonic day 9–10 or within a few hours of birth from massive hemorrhage [21], and by the lack of patients with complete gene deletions [2]. Moreover, the expression of a minimal FV activity because of the introduction of a liver-specific transgene, below the sensitivity threshold of the detection assay (<0.1%), leads to the survival of mice [22].

Gene structure and mutations

The human *F5* gene (GenBank accession *n.* ENSG00000198734) is more than 80 kb long and is located on the long arm of chromosome 1q23. The genes for coagulation FV and FVIII are homologous both in structure and organization, suggesting their evolution from a common ancestral gene [6]. The *F5* gene coding sequence is divided into 25 exons ranging in size from 72 to 2820 base pairs (bp) and 24 introns varying between 0.4 kb and 11 kb. The sequence encoding the large B domain is contained within exon 13. *F5* cDNA is 6914 bp of length, corresponding to a coding region of 6674 bp, 91 bp of 5′ untranslated region and 142 bp of 3′ untranslated region. A total of 83 bp encode the 28-amino-acid hydrophobic signal peptide while in the protein of 2196 amino acids, 709 amino acids form the heavy chain region, 836 amino acids form the connecting region, and 651 amino acids form the light chain region. The nucleotide sequence flanking the initiator ATG codon (AGCATGT) is very similar to the consensus Kozac translation initiator sequence. At the 3′ end of the cDNA the polyadenilation signal sequence (AATAAA) is located 12 nucleotides preceding the poly(A) tail [23].

Very recently, analysis of the *F5* splicing pattern in HepG2 cells and human liver evidenced the occurrence of multiple alternative splicing (AS) events [24]. In particular, among different unproductive ASs, three major ones were detected: two determining the synthesis of in-frame splicing variants and one producing an out-of-frame transcript. The latter was demonstrated to be downregulated by the nonsense-mediated mRNA decay (NMD) pathway, a mechanism that selectively detects and degrades transcripts carrying premature termination codons [25]. This suggests a possible role of the AS-NMD coupling mechanism in the regulation the *F5* gene expression [24].

Human FV deficiency is an autosomal recessive bleeding disorder, characterized by low levels of FV associated with bleeding symptoms ranging from mild to severe [26]. In most of the affected individuals the phenotype is characterized by the concomitant deficiency of factor V activity and antigen (type I deficiency); however, about 25% of the patients have normal antigen levels (type II deficiency), thus indicating that there is a dysfunctional protein [27].

Studies of the molecular basis of severe FV deficiency first took place in 1998 along with the identification of the first causative mutation [28]. However, presumably because of the large size and complexity of the gene, relatively little information is still available.

Until now, a total of 56 mutations have been published as responsible for FV deficiency; more than two-thirds of them are null mutations [small deletions, frame-shift (FS), nonsense, and splice site mutations], mainly decreasing FV expression; the remaining are missense mutations usually impairing FV secretion or accelerating its degradation (Figure 50.1) (ref. 29, last release available on request at H.L.Vos@lumc.nl).

A total of 48 described mutations (21 small insertions/deletions, 14 missense mutations, seven splicing defects, and six nonsense mutations), lead to severe type 1 FV deficiency, only one being recurrent (Tyr1702Cys, repeatedly found in Italian individuals) [30]. This confirms the remarkable allelic heterogeneity of the disease. As for mild type 1 FV deficiency, besides the recurrent Tyr1702Cys mutation, seven additional genetic defects have been, until now, reported in heterozygous index patients: three nonsense, two missense, one splicing, and one insertion/deletion mutations (this survey does not take into account heterozygous relatives of severe FV-deficient patients). A decrease in FV levels, responsible for a mild type 1 deficiency, has also been associated with two FV variants: a functional polymorphism (Met2120Thr), which was demonstrated

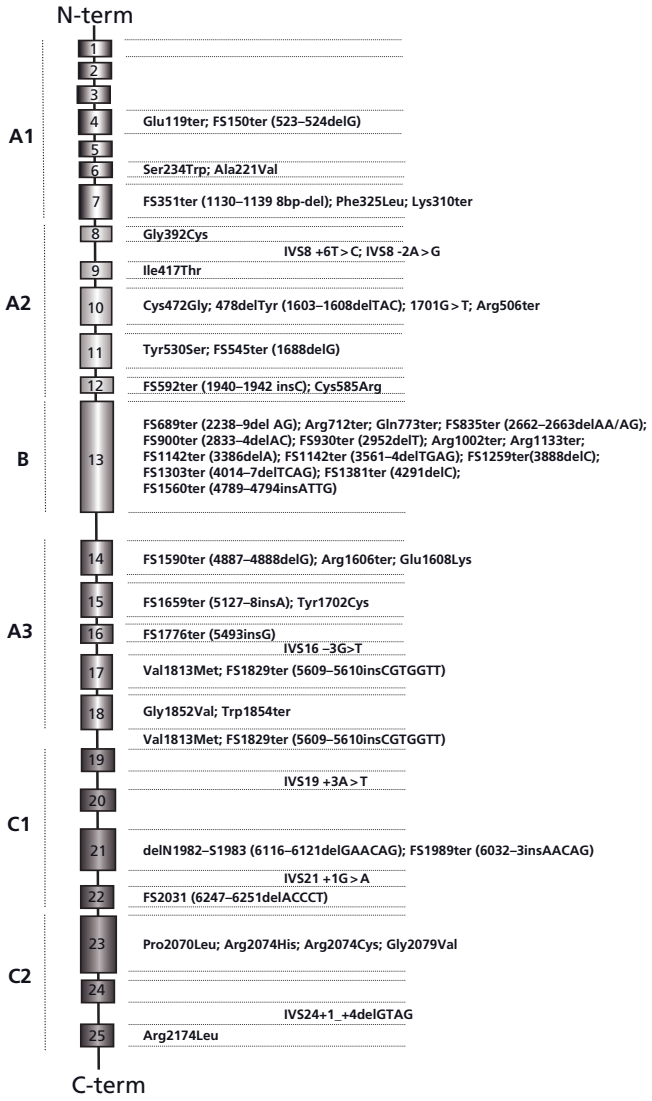

Figure 50.1 Mutations in the *F5* gene, projected on the exons encoding the domains of the protein. Exons and introns are represented by boxes and lines, respectively, and are not drawn to scale. The FV domain structure is also indicated. Only fully published mutations are listed.

to cause an approximate 25% reduction in FV levels [31], and the HR2 haplotype. HR2 is an FV allele defined by a group of more than 10 polymorphisms [31]; among them, the dominant contribution of the Asp2194Gly variant was demonstrated by expression experiments in eukaryotic cells [32]. The first to be described, FV-New Brunswick (Ala221Val), is the only genetic defect associated with type 2 deficiency [33]. The expression of the recombinant FV-Ala221Val demonstrated that the mutation interferes with the stability of mutant FVa [34]. Summing up, mutations causing FV deficiency do not cover the full spectrum of possible genetic lesions; in fact, mutations located in the promoter (or other regulatory regions), as well as large deletions, are absent. Recently, a patient has been described with an FV level of 9%, who had a complete deletion of one FV allele in association with a 1q deletion on one chromosome combined with a point mutation in the other FV allele [35]. Moreover, an uneven distribution of mutations is observable: most genetic defects are present in exons 8 to 25 and missense mutations are completely absent in the large exon 13, coding for the whole B domain. This can probably be explained by an increased "tolerance" of this domain to variations, as suggested by the fact that it is highly polymorphic and not evolutionarily conserved.

Clinical manifestations

Generally, bleeding symptoms develop during the first 6 years of life, and even bleeding from the umbilical stump has been reported [36]. Frequent symptoms are epistaxis and menorrhagia, which occur in approximately 50% of patients, as well as postoperative and oral cavity hemorrhages [26] (see also www.rbdd.org). Other less common symptoms include hemarthroses and hematomas, whereas life-threatening bleeding episodes in the gastrointestinal tract and in the central nervous system are rare [36] (see also www.rbdd.org).

The FV activity level has limited correlation with the severity of bleeding. Overall, patients with lower levels are more likely to have bleeding episodes than those with higher levels. Patients who come to medical attention are typically symptomatic homozygotes or compound heterozygotes with FV activity levels less than 5%, although patients who are compound heterozygotes with levels that ranged from 24% to 68% had no bleeding symptoms [33,37] (see also FactorV-gene-mutations-table-27sept2006. DOC: http://www.lumc.nl/4010/research/factor_V_gene.html).

However, the severity of the clinical phenotype cannot be easily predicted by the activity level. Patients with identical mutations or activity levels, including related patients with identical genotypes and equally low (<1%) FV activities, can vary greatly in their bleeding symptoms [2,30].

Treatment

The choice of dosages and modalities of treatment of bleeding episodes is based on the type of bleeding, on FV levels of affected individuals, and on FV plasma half-life (36 h). Replacement therapy of FV can be administered only through fresh-frozen plasma (FFP), preferably virus-inactivated, since FV concentrates are not available and FV is not present in cryoprecipitate or prothrombin complex concentrates [38]. FV levels should be raised to at least 25 IU/dL [39] by using 15–20 mL/kg FFP [26]. The initial dose should be 15–20 mL/kg followed by smaller amounts, such as 5 mL/kg every 12 h,

adjusting the dosage on the basis of FV levels, PT, and PTT. Studies of FV recovery recommend maintaining a level of 20–25% of FV activity for surgery or in case of severe bleeding [39]. Surgical procedures should be addressed by administering FFP once a day to achieve minimum levels of FV of 25 IU/dL until wound healing is established [39].

It has been suggested that in cases of severe bleeding not controlled with FFP replacement, or in case of inhibitor development, platelet transfusions may be considered. A case of severe FV deficiency associated with multiple episodes of intracranial bleeding at birth was reported, where the inhibitor development owing to FFP infusion was solved by additional administration of platelets [40]. Platelets provide a concentrated supply of FV (approximately 20% of total circulating FV). Therefore, following α-granule release upon platelet activation, FV can presumably bind immediately to surface receptors optimizing prothrombinase complex activity [39].

Development of alloantibodies to FV in FFP is a potential complication of hereditary FV deficiency [39]. Following FFP replacement therapy, the occurrence of inhibitors, especially transient ones of low level, may not be uncommon [39] and can be neutralized using large amounts of FFP [38]. However, as in the treatment of surgical cases, there are concerns over fluid overload in this situation, and close cardiovascular monitoring is advised. Intravenous immunoglobulin also may be effective in eradicating the FV inhibitor [41]. Platelet infusions have been reported to be effective in acquired FV deficiency [42], but it was reported to be effective in stabilizing a subdural hematoma also in a patient with hereditary FV deficiency, complicated by inhibitors [40]. Use of recombinant activated factor VII (rFVIIa) concentrate was reported at a dosage varied between 80 and 100 μg/kg administrated intravenously; however, the hemostatic efficacy in these patients was various [43,44].

Menorrhagia is a common bleeding symptom in women with severe FV deficiency. Management of this symptom usually includes medical treatment such as antifibrinolytics, desmopressin (DDAVP), oral contraceptives, levonorgestrel intrauterine device and replacement therapy, and surgical treatments, such as endometrial ablation and hysterectomy [45]. However, management of women with FV deficiency requires additional monitoring of the hemostatic parameters and awareness of the increased risk of bleeding with any surgical interventions [45].

Combined deficiency of factor V and factor VIII

Combined deficiency of FV and FVIII (F5F8D, OMIM 227300) is an autosomal recessive bleeding disorder characterized by concomitantly low levels (usually between 5% and 20%) of the two coagulation factors V and VIII [46]. F5F8D is completely separate from FV deficiency and FVIII deficiency. The latter two are transmitted with different patterns of inheritance (autosomal recessive for FV, X-linked for FVIII) and involve proteins encoded by two different genes (*F5* gene and *F8* gene). F5F8D was first described by Oeri *et al.* in 1954 [47]. However, the molecular mechanism of the association of the combined factor deficiency was not understood until 1998, when Nichols *et al.* [48,49] discovered that the cause of the deficiency was associated with null mutations in the *ERGIC-53* gene, now called *LMAN1* gene (lectin mannose binding protein), encoding an endoplasmic reticulum (ER)–Golgi intermediate compartment (ERGIC) marker protein. Mutations in *LMAN1* were found in ~70% of affected patients but 30% of this population had no detectable mutation in *LMAN1*. In 2003, Zhang *et al.* [50] identified a second locus associated with the deficiency in about 15% of affected families with no mutation in *LMAN1*. The *MCFD2* (multiple coagulation factor deficiency 2) gene encodes for a cofactor for LMAN1 [51]. Even if a debate existed on the possible existence of other loci involved in the intracellular transport of FV and FVIII and associated with the disease, until now previous biochemical studies failed to identify additional components of the LMAN1–MCFD2 receptor complex [51], supporting the idea that F5F8D might be limited to the *LMAN1* and *MCFD2* genes [52].

Congenital F5F8D is estimated to be extremely rare (1 : 1 000 000) in the general population [9]. However, this disorder was reported to be particularly prevalent among Middle Eastern Jewish and non-Jewish Iranians, where the incidence was estimated at ~1 : 100 000 [53]. This high frequency is probably owing, at least in part, to the high incidence of consanguineous marriages in these populations [54]. Cases were reported from different countries principally belonging to Europe, the Middle East, Asia, Africa, and America [55].

The worldwide prevalence of F5F8D patients, as indicated by the WFH 2007 global survey and the RBDD (www.rbdd.org) seem to be ~3% of the total number of patients affected by RBDs, indicating that F5F8D is one of the rarest coagulation disorders.

LMAN1 and MCFD2 proteins

LMAN1 is a 53-kDa type 1 transmembrane nonglycosylated protein with homology to leguminous lectin proteins [56]. It displays different oligomerization states—monomer, dimer, and hexamer—which have been implicated in its exit/retention within the ER, and it is thought to bind correctly folded glycosylated cargo proteins, including FV and FVIII in the ER, recruiting the cargo for package into coat protein complex II (COPII)-coated vesicles and to transport them first to the ERGIC and then to the Golgi [57]. Indeed, LMAN1 resides in the early secretory pathway, with highest concentration in the ERGIC compartment. LMAN1 consists of a luminal, a

transmembrane and a short cytoplasmatic domain for a total of 513 residues. The luminal domain can be divided into two subdomains, an N-terminal carbohydrate recognition domain (CRD) (residues 31–285) and a membrane-proximal α-helical coiled domain, the stalk domain (residues 290–460).

The crystal structures of the CRD in apo- and Ca^{2+}-bound form of LMAN1 were determined [58,59]. The CRD is responsible for the calcium ion-dependent binding of the protein to mannose-rich glycans. The stalk domain, which is predicted to be a coiled-coil structure, contains two cysteines thought to mediate oligomerization of the protein through disulfide interactions [60,61] while the cytoplasmic tail binds to the COPII component of vesicle coats, allowing efficient ER export; it also includes a retrieval sequence to bring the transporter back to the ER after release of its cargo in the ERGIC [62]. Efficient transport of coagulation FV and FVIII along the secretory pathway requires the integrity of their heavily glycosylated B domains and a functional LMAN1 protein, and an interaction between FVIII and LMAN1 has been demonstrated [51,63]. LMAN1 has thus been implicated to act as a sorting receptor, mediating transport of certain glycoproteins from the ER to Golgi. In support of such an important function, homologues of LMAN1 have been identified in levels of the animal kingdom ranging from *Caenorhabditis elegans* to man and display a high degree of sequence identity [64].

MCFD2 (multiple coagulation factor deficiency 2) is a small (146 residues) soluble protein of 16 kDa with a signal sequence mediating translocation into the ER and two EF-hand motifs that may bind Ca^{2+} ions in the C-terminal region. MCFD2 forms a Ca^{2+}-dependent 1:1 stoichiometric complex with LMAN1, that works as a cargo receptor for efficient ER–Golgi transfer of coagulation FV and FVIII during their secretion [50]. While, to date, several proteins have been identified as cargo of LMAN1 (FV, FVIII, cathepsin C, cathepsin Z, nicastrin, and α1-antitrypsin) [65–68], MCFD2 is only known to be required for transport of the blood coagulation factors, suggesting a possible role for MCFD2 as a specific recruitment factor for this subset of LMAN1 cargo proteins [69]. Interestingly, an MCFD2 mutant which fails to coimmunoprecipitate with LMAN1 has been shown to retain the ability to interact with FVIII, implying that the interaction between MCFD2 and the coagulation factors may be independent of MCFD2/LMAN1 binding [51]. Unlike LMAN1, MCFD2 does not include the Phe–Phe (FF) motif required for COPII binding or the Lys–Lys (KK) motif which functions as an ER retrieval signal, suggesting that correct localization of MCFD2 is reliant on its interaction with LMAN1 [50]. The crystal structure of the MCFD2 in solution was recently determined by NMR by Guy *et al.* [57], reporting that MCFD2 is disordered in the absence of calcium, while it adopts a predominantly ordered (folded) structure on binding calcium, leaving the N-terminus disordered and the extended loop between the two EF-hand motifs with conformational flexibility [57].

Genes structure and mutations

LMAN1 is encoded by a gene of approximately 29 kb located on chromosome 18q21 and containing 13 exons [70]. MCFD2 is encoded by a gene of approximately 19 kb located on chromosome 2p21 and containing four exons [50].

To date, 48 mutations in *LMAN1* (n = 32, 67%) and *MCFD2* (n = 16, 33%) genes have been described [71–75] (see also http://www.isth.org/Publications/RegistriesDatabases/MutationsRareBleedingDisorders/CombinedFactorVandVIIIdeficiency.aspx) (Figures 50.2 and 50.3). All the *LMAN1* mutations reported to date are null mutations with the exception of a cysteine-to-arginine mutation that disrupts a disulfide bond that is required for its oligomerization and also destabilizes the protein [52] (Figure 50.2). In contrast, both null mutations and missense mutations have been identified in *MCFD2* (Figure 50.3). Five of the six *MCFD2* missense mutations reported to date [50,71,72] have been shown to disrupt LMAN1 binding, indicating that LMAN1 and MCFD2 must function as a unit to transport FV and FVIII. Although

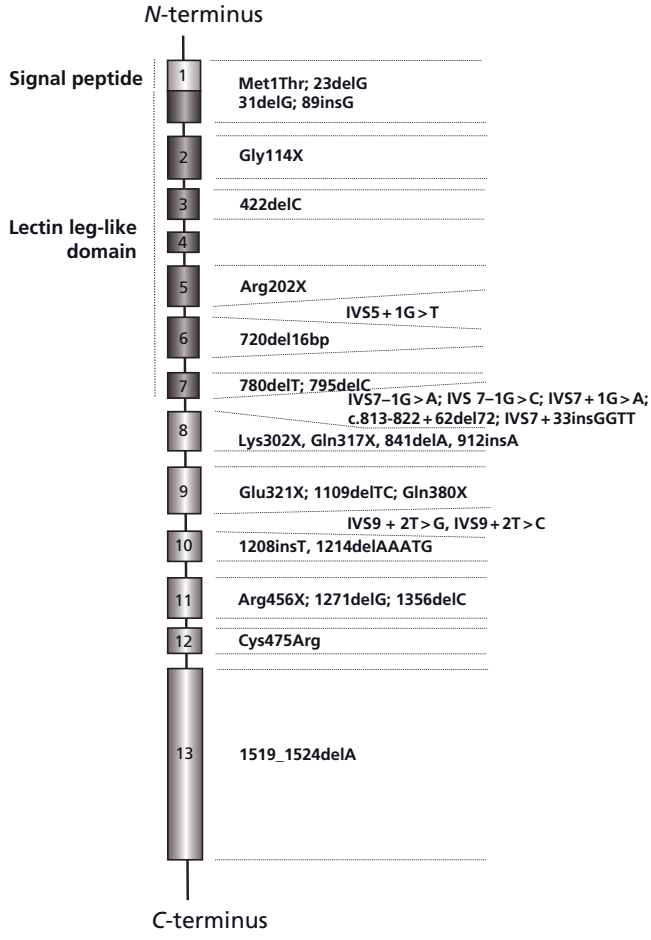

Figure 50.2 Mutation in the *LMAN1* gene, projected on the exons encoding the domains of the protein. Exons and introns are represented by boxes and lines, respectively, and are not drawn to scale. Only fully published mutations are listed.

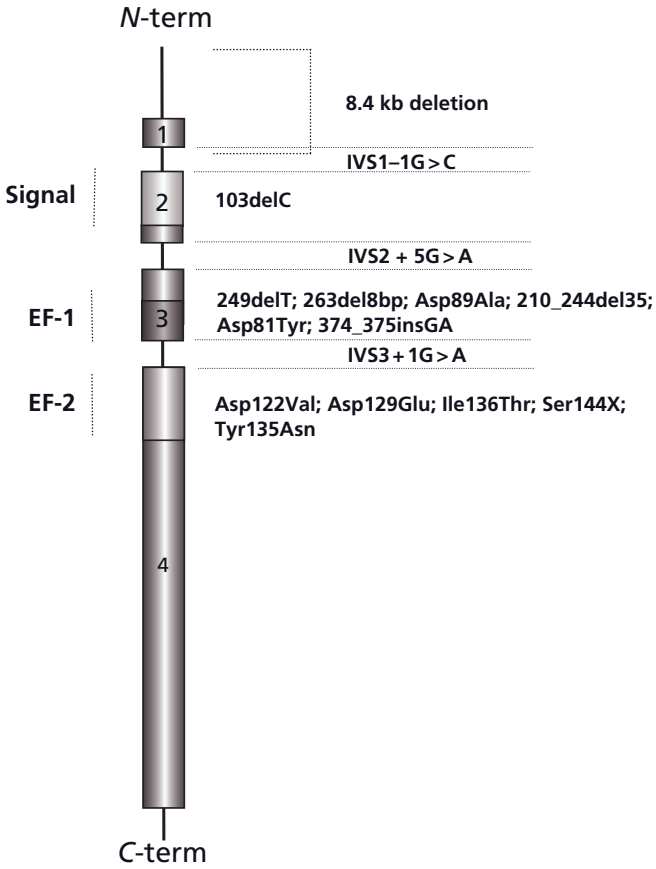

Figure 50.3 Mutation in the *MCFD2* gene, projected on the exons encoding the domains of the protein. Exons and introns are represented by boxes and lines, respectively, and are not drawn to scale. Only fully published mutations are listed.

MCFD2 appears to directly interact with FVIII [51], it is unclear whether LMAN1 binds directly to FV/FVIII or indirectly via MCFD2.

Mutations in *LMAN1* and *MCFD2* are associated with indistinguishable phenotypes [50]. However, a selective delay in secretion of the protein procathepsin C has been observed in HeLa cells overexpressing a dominant negative form of LMAN1 [66]. These results were confirmed by Nyfeler *et al*., showing that LMAN1 also interacts with the two lysosomal glycoproteins cathepsin Z and cathepsin C whereas MCFD2 is dispensable for the binding of them to LMAN1 [69]. Zhang *et al*. [72] recently performed a genotype–phenotype analysis by combining their data together with all previously published reports to evaluate if mutations in different genes are associated with differences in the FV and FVIII plasma levels. A difference between these two classes of patients (those carrying *LMAN1* or *MCFD2* mutations) in the distribution of plasma levels for FV and FVIII was found: the mean levels of plasma FV and FVIII in patients with *MCFD2* mutations were significantly lower than the corresponding levels in patients with *LMAN1* mutations. These data suggest that MCFD2 may play a primary role in the export of FV and FVIII from the ER, with the impact of LMAN1 being mediated indirectly through its interaction with MCFD2 [72].

Some of the identified mutations in both the *LMAN1* and *MCFD2* genes are recurrent in more than one family. *LMAN1* mutations, such as *p*.M1T, c.86_89insG, *p*.R202X, c.822G>A, *p*.K302X, and c.1149+2T>C, have been observed in more than four patients [49,54,76–79]. Similarly, the c.149+5G>A and *p*.I136T mutations in *MCFD2* have also been commonly reported [72,73,79]. The *MCFD2* c.149+5G>A appears to be one of the most common mutations causing F5F8D and it has now been identified in at least 13 unrelated families from different geographic regions and particularly from India (n = 6) [80,73], followed by Italy (n = 4) [52,72], USA (n = 1) [50], Serbia (n = 1) [52], and Germany (n = 1) [50].

In vitro expression studies have proved to be an invaluable tool to understand the nature of the genetic defect and to unravel the underlying molecular mechanism of deficiencies. *In vitro* expression studies of *LMAN1* and *MCFD2* gene mutations and characterization of the activity of the corresponding recombinant proteins in the secretion of coagulation FV and FVIII could thus significantly help to describe the mechanism of the deficiency. In the literature, five out of the six identified *MCFD2* missense mutations (D129E, I136T, D81Y, D89A, and D122V) result in an amino acid substitution at a highly conserved amino acid residue. All these mutations are in one of the two EF-hand domains. They were expressed in COS-1 transfected cells [50,57,72,81], and have been shown to disrupt LMAN1 binding. All the other identified mutations were demonstrated to be associated with the deficiency through analysis performed on Epstein–Barr virus (EBV)-transformed lymphoblasts of the patients that showed the presence or absence of the mutated protein [50,52,76].

Clinical manifestations

F5F8D is characterized by concomitantly low levels (usually between 5% and 20%) of the two coagulation factors, FV and FVIII, both as coagulant activity and antigen [46]. The concomitant presence of two coagulation defects does not enhance the hemorrhagic tendency that was observed in each defect separately [82,83]. F5F8D is associated with a mild to moderate bleeding tendency [9,53–54,71,82–84] (see also www.rbdd.org). Mild bleeding symptoms such as easy bruising, epistaxis, and gum bleeding are not uncommon in affected individuals. Other common bleedings include soft-tissue hematomas [9,85], bleeding after surgery, dental extraction and trauma, and menorrhagia and postpartum hemorrhage in affected women [53,83]. In F5F8D patients, circulating levels of FV and FVIII are usually sufficient to prevent more severe spontaneous bleeding episodes [39,53,83]. However, more severe symptoms, such as hemarthroses, could be observed. Other severe symptoms, such as gastrointestinal and central nervous system bleedings, were reported in only a few patients

[82,83]. Excessive bleeding after circumcision was reported in two-thirds (8/12) and approximately half (6/13) of the male patients [82,83].

Treatment

In F5F8D, bleeding episodes are usually treated on demand and do not require regular prophylaxis [39]. Treatment of bleeding episodes is generally chosen according to the nature of the bleed and the FV and FVIII levels of affected individuals. Both FV and FVIII sources are needed and their plasma half-life (FV: 36 h; FVIII: 10–14 h) have to be taken into consideration.

As there are no FV concentrates available and FV is not present in cryoprecipitate or prothrombin complex concentrates [38], replacement of FV could be achieved only through the use of FFP, preferably with virus-inactivated plasma. For FVIII replacement, a large number of products are available, including FFP, plasma-derived concentrates, or recombinant FVIII (rFVIII) (different generations).

Factor V replacement

See section on factor V deficiency.

Factor VIII replacement

FVIII levels should be raised to at least 30–50 IU/dL for the treatment of minor bleeding episodes and to at least 50–70 IU/dL for more severe bleeds. The synthetic hormone desmopressin (DDAVP, 260 mg intranasal or 0.3 mg/kg subcutaneous), can be successfully used for minor bleeding episodes to further raise FVIII [39]. However, its efficacy needs to be tested in each patient and more than three to four consecutive treatments should be avoided [9]. For severe bleedings, plasma-derived FVIII or rFVIII concentrates are the treatments of choice. Surgical procedures should be addressed by administering FVIII 30 min before surgery and then every 12 h to maintain FVIII levels above 50 IU/dL [39].

Prenatal diagnosis

In developing countries, where management is still largely inadequate, patients with rare bleeding disorders rarely live beyond childhood. Thus, molecular characterization, carrier detection, and prenatal diagnosis remain the key steps for the prevention of the birth of children affected by coagulation disorders or for the preparation of on-demand treatment in case of neonatal hemorrhages. As for FV deficiency, though life-threatening episodes at birth are rare, the use of prenatal diagnosis can be advisable only in those families with severe clinical history, presenting affected members. Since patients with F5F8D have a mild to moderate bleeding tendency, prenatal diagnosis is not currently performed and not recommended.

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References

1 Dahlback B. Blood coagulation. *Lancet* 2000; **355**: 1627–32.
2 Kalafatis M. Coagulation factor V: a plethora of anticoagulant molecules. *Curr Opin Hematol* 2005; **12**: 141–8.
3 Asselta R, Tenchini ML, Duga S. Inherited defects of coagulation factor V: the hemorrhagic side. *J Thromb Haemost* 2006; **4**: 26–34.
4 Kingsley CS. Familial factor V deficiency: the pattern of heredity. *Q J Med* 1954; **23**: 323–9.
5 Jenny RJ, Pittman DD, Toole JJ, *et al.* Complete cDNA and derived amino acid sequence of human factor V. *Proc Natl Acad Sci USA* 1987; **84**: 4846–50.
6 Cripe LD, Moore KD, Kane WH. Structure of the gene for human coagulation factor V. *Biochemistry* 1992; **31**: 3777–85.
7 Janeway CM, Rivard GE, Tracy PB, *et al.* Factor V Quebec revisited. *Blood* 1996; **87**: 3571–8.
8 Streiff MB, Ness PM. Acquired FV inhibitors: a needless iatrogenic complication of bovine thrombin exposure. *Transfusion* 2002; **42**: 18–26.
9 Mannucci PM, Duga S, Peyvandi F. Recessively inherited coagulation disorders. *Blood* 2004; **104**: 1243–52.
10 Mann KG, Kalafatis M. Factor V: a combination of Dr Jekyll and Mr Hyde. *Blood* 2003; **101**: 23–30.
11 Kane WH, Davie EW. Blood coagulation factor V and VIII: structural and functional similarities and their relationship to hemorrhagic and thrombotic disorders. *Blood* 1988; **71**: 539–55.
12 Ortel TL, Takahashi N, Putnam FW. Structural model of human ceruloplasmin based on internal triplication, hydrophilic/hydrophobic character, and secondary structure of domains. *Proc Nat Acad Sci USA* 1984; **81**: 4761–65.
13 Baumgartner S, Hofmann K, Chiquet-Ehrismann R, *et al.* The discoidin domain family revisited: new members from prokaryotes and a homology-based fold prediction. *Protein Sci* 1998; **7**: 1626–31.
14 Macedo-Ribeiro S, Bode W, Huber R, *et al.* Crystal structures of the membrane-binding C2 domain of human coagulation factor V. *Nature* 1999; **402**: 434–9.
15 Adams TE, Hockin MF, Mann KG, *et al.* The crystal structure of activated protein C inactivated bovine factor Va: implications for cofactor function. *Proc Natl Acad Sci USA* 2004; **101**: 8918–23.
16 Stoilova-McPhie S, Parmenter CD, Segers K, *et al.* Defining the structure of membrane-bound human blood coagulation factor Va. *J Thromb Haemost* 2008; **6**: 76–82.
17 Lee CJ, Lin P, Chandrasekaran V, *et al.* Proposed structural models of human factor Va and prothrombinase. *J Thromb Haemost* 2008; **6**: 83–9.
18 Foster WB, Nesheim ME, Mann KG. The factor Xa-catalyzed activation of factor V. *J Biol Chem* 1983; **258**: 13970–77.
19 Huang JN, Koerper MA. Factor V deficiency: a concise review. *Haemophilia* 2008; **14**: 1164–9.
20 Kalafatis M, Rand MD, Mann KG. The mechanism of inactivation of human factor V and human factor Va by activated protein C. *J Biol Chem* 1994; **269**: 31869–80.

21 Cui J, O'Shea KS, Purkayastha A, *et al.* Fatal haemorrhage and incomplete block to embryogenesis in mice lacking coagulation factor V. *Nature* 1996; **384**: 66–8.

22 Yang TL, Cui J, Taylor JM, *et al.* Rescue of fatal neonatal hemorrhage in factor V deficient mice by low transgene expression. *Thromb Haemost* 2000; **83**: 70–7.

23 Tuddenham EGD, Cooper DN. *The Molecular Genetics of Hemostasis and its Inherited Disorders*. Oxford: Oxford University Press, 1994.

24 Dall'osso C, Guella I, Duga S, *et al.* Molecular characterization of three novel splicing mutations causing factor V deficiency and analysis of the F5 gene splicing pattern. *Haematologica* 2008; **93**: 1505–13.

25 Frischmeyer PA, Dietz HC. Nonsense-mediated mRNA decay in health and disease. *Hum Mol Genet* 1999; **10**: 1893–900.

26 Peyvandi F, Duga S, Akhavan S, *et al.* Rare coagulation deficiencies. *Haemophilia* 2002; **8**: 308–21.

27 Chiu HC, Whitaker E, Colman RW. Heterogeneity of human factor V deficiency. Evidence for the existence of an antigen-positive variant. *J Clin Invest* 1983; **72**: 493–503.

28 Guasch JF, Cannegieter S, Reitsma PH, *et al.* Severe coagulation factor V deficiency caused by a 4 bp deletion in the factor V gene. *Br J Haematol* 1998; **101**: 32–9.

29 Vos HL. An online database of mutations and polymorphisms in and around the coagulation factor V gene. *J Thromb Haemost* 2007; **5**: 185–8.

30 Castoldi E, Lunghi B, Mingozzi F, *et al.* A missense mutation (Y1702C) in the coagulation factor V gene is a frequent cause of factor V deficiency in the Italian population. *Haematologica* 2001; **86**: 629–33.

31 Scanavini D, Girelli D, Lunghi B, *et al.* Modulation of factor V levels in plasma by polymorphisms in the C2 domain. *Arterioscler Thromb Vasc Biol* 2004; **24**: 200–6.

32 Yamazaki T, Nicolaes GA, Sorensen KW, *et al.* Molecular basis of quantitative factor V deficiency associated with factor V R2 haplotype. *Blood* 2002; **100**: 2515–21.

33 Murray JM, Rand MD, Egan JO, *et al.* Factor V New Brunswick: Ala221-to-Val substitution results in reduced cofactor activity. *Blood* 1995; **86**: 1820–7.

34 Steen M, Miteva M, Villoutreix BO, *et al.* Factor V New Brunswick: Ala221Val associated with FV deficiency reproduced in vitro and functionally characterized. *Blood* 2003; **102**: 1316–22.

35 Caudill JS, Sood R, Zehnder JL, *et al.* Severe coagulation factor V deficiency associated with an interstitial deletion of chromosome 1q. *J Thromb Haemost* 2007; **5**: 626–8.

36 Lak M, Sharifian R, Peyvandi F, *et al.* Symptoms of inherited factor V deficiency in 25 Iranian patients. *Br J Haematol* 1998; **103**: 1067–9.

37 Kingsley CS. Familial factor V deficiency: the pattern of heredity. *Q J Med* 1954; **23**: 323–9.

38 Di Paola J, Nugent D, Young G. Current therapy for rare factor deficiency. *Haemophilia* 2001; **7**: 16–22.

39 Bolton-Maggs PH, Perry DJ, Chalmers EA, *et al.* The rare coagulation disorders—review with guidelines for management from the United Kingdom Haemophilia Centre Doctors' Organisation. *Haemophilia* 2004; **10**: 593–628.

40 Salooja N, Martin P, Khair K, *et al.* Severe factor V deficiency and neonatal intracranial haemorrhage: a case report. *Haemophilia* 2000; **6**: 44–6.

41 Tarantino MD, Ross MP, Daniels TM, *et al.* Modulation of an acquired coagulation factor V inhibitor with intravenous immune globulin. *J Pediatr Hematol Oncol* 1997; **19**: 226–31.

42 Chediak J, Ashenhurst JB, *et al.* Successful management of bleeding in a patient with factor V inhibitor by platelet transfusions. *Blood* 1980; **56**: 835–41.

43 Altisent C, Lozano M, Sol E, *et al.* Dental extraction using rFVIIa in a patient with severe FV deficiency. *Haemophilia* 2000; **6**: 408.

44 González-Boullosa R, Ocampo-Martínez R, Alarcón-Martín MJ, *et al.* The use of activated recombinant coagulation factor VII during haemarthroses and synovectomy in a patient with congenital severe factor V deficiency. *Haemophilia* 2005; **11**: 167–70.

45 Lee CA, Chi C, Pavord SR, *et al.* The obstetric and gynaecological management of women with inherited bleeding disorders—review with guidelines produced by a taskforce of UK Haemophilia Centre Doctors Organization. *Haemophilia* 2006; **12**: 301–36.

46 Giddings JC, Seligsohn U, Bloom AL. Immunological studies in combined factor V and factor VIII deficiency. *Br J Haematol* 1977; **37**: 257–64.

47 Oeri J, Matter M, Isenschmid H, *et al.* Congenital factor V deficiency (parahemophilia) with true hemophilia in two brothers. *Bibl Paediatr* 1954; **58**: 575–88.

48 Nichols WC, Seligsohn U, Zivelin A, *et al.* Linkage of combined factors V and VIII deficiency to chromosome 18q by homozygosity mapping. *J Clin Invest* 1997; **99**: 596–601.

49 Nichols WC, Seligsohn U, Zivelin A, *et al.* Mutations in the ER–Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of coagulation factors V and VIII. *Cell* 1998; **93**: 61–70.

50 Zhang B, Cunningham MA, Nichols WC, *et al.* Bleeding due to disruption of a cargo-specific ER-to-Golgi transport complex. *Nat Genet* 2003; **34**: 220–5.

51 Zhang B, Kaufman RJ, Ginsburg D. LMAN1 and MCFD2 form a cargo receptor complex and interact with coagulation factor VIII in the early secretory pathway. *J Biol Chem* 2005; **280**: 25881–6.

52 Zhang B, McGee B, Yamaoka JS, *et al.* Combined deficiency of factor V and factor VIII is due to mutations in either LMAN1 or MCFD2. *Blood* 2006; **107**: 903–7.

53 Seligsohn U, Zivelin A, Zwang E. Combined factor V and factor VIII deficiency among non-Ashkenazi Jews. *N Engl J Med* 1982; **307**: 1191–5.

54 Segal A, Zivelin A, Rosenberg N, *et al.* A mutation in LMAN1 (ERGIC-53) causing combined factor V and factor VIII deficiency is prevalent in Jews originating from the island of Djerba in Tunisia. *Blood Coagul Fibrinolysis* 2004; **15**: 99–102.

55 Spreafico M, Peyvandi F. Combined FV and FVIII deficiency. *Haemophilia* 2008; **14**: 1201–8.

56 Itin C, Roche AC, Monsigny M, *et al.* ERGIC-53 is a functional mannose-selective and calcium-dependent human homologue of leguminous lectins. *J Cell Biol* 1996; **107**: 483–93.

57 Guy JE, Wigren E, Svärd M, *et al.* New insights into multiple coagulation factor deficiency from the solution structure of human MCFD2. *J Mol Biol* 2008; **381**: 941–55.

58 Velloso LM, Svensson K, Schneider G, *et al.* Crystal structure of the carbohydrate recognition domain of p58/ERGIC-53, a protein involved in glycoprotein export from the endoplasmic reticulum. *J Biol Chem* 2002; **277**: 15979–84.

59 Velloso LM, Svensson K, Pettersson RF, *et al.* The crystal structure of the carbohydrate-recognition domain of the glycoprotein sorting receptor p58/ERGIC-53 reveals an unpredicted metal-binding site and conformational changes associated with calcium ion binding. *J Mol Biol* 2003; **334**: 845–51.

60 Lahtinen U, Svensson K, Pettersson RF. Mapping of structural determinants for the oligomerization of p58, a lectin-like protein

of the intermediate compartment and cis-Golgi. *Eur J Biochem* 1999; **260**: 392–7.
61 Nufer F, Kappeler S, Guldbrandsen S, *et al.* ER export of ERGIC-53 is controlled by cooperation of targeting determinants in all three of its domains. *J Cell Sci* 2003; **116**: 4429–40.
62 Tisdale EJ, Plutner H, Matteson J, *et al.* p53/58 binds COPI and is required for selective transport through the early secretory pathway. *J Cell Biol* 1997; **137**: 581–93.
63 Cunningham MA, Pipe SW, Zhang B, *et al.* LMAN1 is a molecular chaperone for the secretion of coagulation factor VIII. *J Thromb Haemost* 2003; **1**: 2360–7.
64 Wen C, Greenwald I. p24 proteins and quality control of LIN-12 and GLP-1 trafficking in *Caenorhabditis elegans*. *J Cell Biol* 1999; **145**: 1165–75.
65 Appenzeller C, Andersson H, Kappeler F, *et al.* The lectin ERGIC-53 is a cargo transport receptor for glycoproteins. *Nat Cell Biol* 1999; **1**: 330–4.
66 Vollenweider F, Kappeler F, Itin C, *et al.* Mistargeting of the lectin ERGIC-53 to the endoplasmic reticulum of HeLa cells impairs the secretion of a lysosomalenzyme. *J Cell Biol* 1998; **142**: 377–89.
67 Nyfeler B, Reiterer V, Wendeler MW, *et al.* Identification of ERGIC-53 as an intracellular transport receptor of alpha1-antitrypsin. *J Cell Biol* 2008; **180**: 705–12.
68 Morais VA, Brito C, Pijak DS, *et al.* N-glycosylation of human nicastrin is required for interaction with the lectins from the secretory pathway calnexin and ERGIC-53. *Biochim Biophys Acta* 2006; **1762**: 802–10.
69 Nyfeler B, Zhang B, Ginsburg D, *et al.* Cargo selectivity of the ERGIC-53/MCFD2 transport receptor complex. *Traffic* 2006; **7**: 1473–81.
70 Neerman-Arbez M, Antonarakis SE, Blouin JL, *et al.* The locus for combined factor V–factor VIII deficiency (F5F8D) maps to 18q21, between D18S849 and D18S1103. *Am J Hum Genet* 1997; **61**: 143–50.
71 Ivaskevicius V, Windyga J, Baran B, *et al.* The first case of combined coagulation factor V and coagulation factor VIII deficiency in Poland due to a novel p.Tyr135Asn missense mutation in the MCFD2 gene. *Blood Coagul Fibrinolysis* 2008; **19**: 431–4.
72 Zhang B, Spreafico M, Zheng C, *et al.* Genotype–phenotype correlation in combined deficiency of factor V and factor VIII. *Blood* 2008; **111**: 5592–600.
73 Jayandharan G, Spreafico M, Viswabandya A, *et al.* Mutations in the *MCFD2* gene are predominant among patients with hereditary combined FV and FVIII deficiency (F5F8D) in India. *Haemophilia* 2007; **13**: 413–19.
74 Ma ES, Wong CL, Lam HY, *et al.* Combined factors V and VIII deficiency (F5F8D) in a Chinese family due to compound heterozygosity for nonsense mutations of the *LMAN1* gene. *Br J Haematol* 2007; **139**: 509–11.
75 Nyfeler B, Kamiya Y, Boehlen F, *et al.* Deletion of 3 residues from the C-terminus of MCFD2 affects binding to ERGIC-53 and causes combined factor V and factor VIII deficiency. *Blood* 2008; **111**: 1299–301.
76 Neerman-Arbez M, Johnson KM, Morris MA, *et al.* Molecular analysis of the ERGIC-53 gene in 35 families with combined factor V-factor VIII deficiency. *Blood* 1999; **93**: 2253–60.
77 Nichols WC, Valeri HT, Wheatley MA, *et al.* ERGIC-53 gene structure and mutation analysis in 19 combined factors V and VIII deficiency families. *Blood* 1999; **93**: 2261–6.
78 D'Ambrosio R, Santacroce R, Di Perna P, *et al.* A new case of combined factor V and factor VIII deficiency further suggests that the LMAN1 M1T mutation is a frequent cause in Italian patients. *Blood Coagul Fibrinolysis* 2007; **18**: 203–4.
79 Dansako H, Ishimaru F, Takai Y, *et al.* Molecular characterization of the *ERGIC-53* gene in two Japanese patients with combined factor V–factor VIII deficiency. *Ann Hematol* 2001; **80**: 292–4.
80 Mohanty D, Ghosh K, Shetty S, *et al.* Mutations in the *MCFD2* gene and a novel mutation in the *LMAN1* gene in Indian families with combined deficiency of factor V and VIII. *Am J Hematol* 2005; **79**: 262–6.
81 Gifford JL, Walsh MP, Vogel HJ. Structures and metal-ion-binding properties of the Ca^{2+}-binding helix-loop-helix EF-hand motifs. *Biochem J* 2007; **405**: 199–221.
82 Mansouritorgabeh H, Rezaieyazdi Z, Pourfathollah AA, *et al.* Haemorrhagic symptoms in patients with combined factors V and VIII deficiency in north-eastern Iran. *Haemophilia* 2004; **10**: 271–5.
83 Peyvandi F, Tuddenham EG, Akhtari AM, *et al.* Bleeding symptoms in 27 Iranian patients with the combined deficiency of factor V and factor VIII. *Br J Haematol* 1998; **100**: 773–6.
84 Shetty S, Madkaikar M, Nair S, *et al.* Combined factor V and VIII deficiency in Indian population. *Haemophilia* 2000; **6**: 504–7.
85 Peyvandi F, Mannucci PM. Rare coagulation disorders. *Thromb Haemost* 1999; **82**: 1207–14.

51 Congenital factor VII deficiency

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Introduction

Factor VII [FVII; previous synonyms: proconvertin, stable factor, serum prothrombin conversion accelerator (SPCA)] is a vitamin K-dependent clotting factor belonging to the "extrinsic pathway" of blood coagulation, a pathway which, for a long time, had been considered an alternative to the main one, the "intrinsic pathway." Today, the complex formed between the naturally occurring procoagulant serine protease, activated FVII (FVIIa), and the integral membrane protein tissue factor (TF), exposed on the vascular lumen upon injury, is known to be the trigger of blood clotting [1]. The FVIIa–TF complex further activates the FVII zymogen to FVIIa and generates a burst of activated factors IX (FIXa) and X (FXa), ultimately leading to the formation of a stable fibrin clot [2,3]. In the bloodstream, FVIIa is the active portion of the FVII mass and is detectable in normal concentrations as low as 5–10 ng, i.e., 1–2% of the zymogen; in contrast to FVII zymogen, FVIIa has a very high affinity for TF.

Free FVIIa does not react with any known inhibitor, but the blood coagulation initiation process is modulated by TFPI (tissue factor pathway inhibitor), a lipid-bound naturally occurring inhibitor which involves TF and factors VIIa and Xa in a quaternary complex [3]; another inhibitor is antithrombin, acting on the TF–FVIIa binary complex [4].

FVII deficiency is not believed to be associated with a complete absence of functional FVII, and this fact is in agreement with data from knockout mice studies [5], which suggest that a complete absence of FVII is incompatible with life.

Congenital FVII deficiency is the commonest among the rare inherited bleeding disorders, with an estimated prevalence of 1 in 500 000 individuals [6] and without ethnic or gender predilection for the defect [7–9]. The bleeding disorder was first described by Alexander *et al*. [10] and subsequent series of patients were reported [11–13] that further clarified the clinical picture and the clotting phenotype. Subsequently, numerous mutations underlying this disorder (http://europium.csc.mrc.ac.uk) have been described, which are predictive for a considerable heterogeneity in both the clotting and clinical phenotypes [14,15].

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Ample clinical heterogeneity is the hallmark for this hemorrhagic disorder, which ranges in severity from lethal to mild, or even asymptomatic forms [15]. The molecular genetics, FVII structure/function analysis, and pathophysiology of FVII deficiency have been extensively investigated [14–21].

Comprehensive studies, focused on clinical issues and involving large patient populations, have reported systematic analyses of disease presentation and diagnosis [15]. The relationship between gender and bleeding tendency has also been investigated [15]. It is known that FVII levels are clearly modulated by *F7* gene polymorphisms [22,23], and databases of mutations are available for comparison [14].

Genetics and pathophysiology

The *F7* gene [24] is located on chromosome 13q34, 2.8 kb upstream of the *FX* gene, and contains nine exons (1a, 1b, 2, 3, 4, 5, 6, 7, 8) (Figure 51.1) encoding the FVII protein circulating in plasma as a 406-amino-acid single chain (50 kDa) (vb). FVII activation generates two chains—the light chain, containing the γ-carboxyglutamic acid (Gla) domain and two epidermal growth factor (EGF)-like domains, and the heavy chain, which contains the catalytic domain.

The *F7* gene has been extensively investigated by DNA sequencing of all coding regions, exon–intron boundaries and the promoter region, with sequencing methods that now enable very efficient detection of mutations in patients with FVII deficiency. Moreover, the expression of mutations detected in FVII-deficient patients has been investigated by recombinant FVII molecules obtained in cultured eukaryotic cells transfected with the expression vectors after site-directed mutagenesis of the human FVII cDNA [25].

The processing of mutated FVII molecules has also been investigated by FVII chimeric FVII green fluorescent protein constructs [26].

Mutations of the *F7* gene [14,15] have been characterized in the vast majority of FVII-deficient patients. FVII deficiency is not believed to be associated with complete absence of functional FVII and this fact accords with data from knockout mice studies [5], which suggest that a complete absence of FVII is incompatible with life.

Mutations are very heterogeneous (Figure 51.1) and missense mutations are the most frequent changes (70–80%).

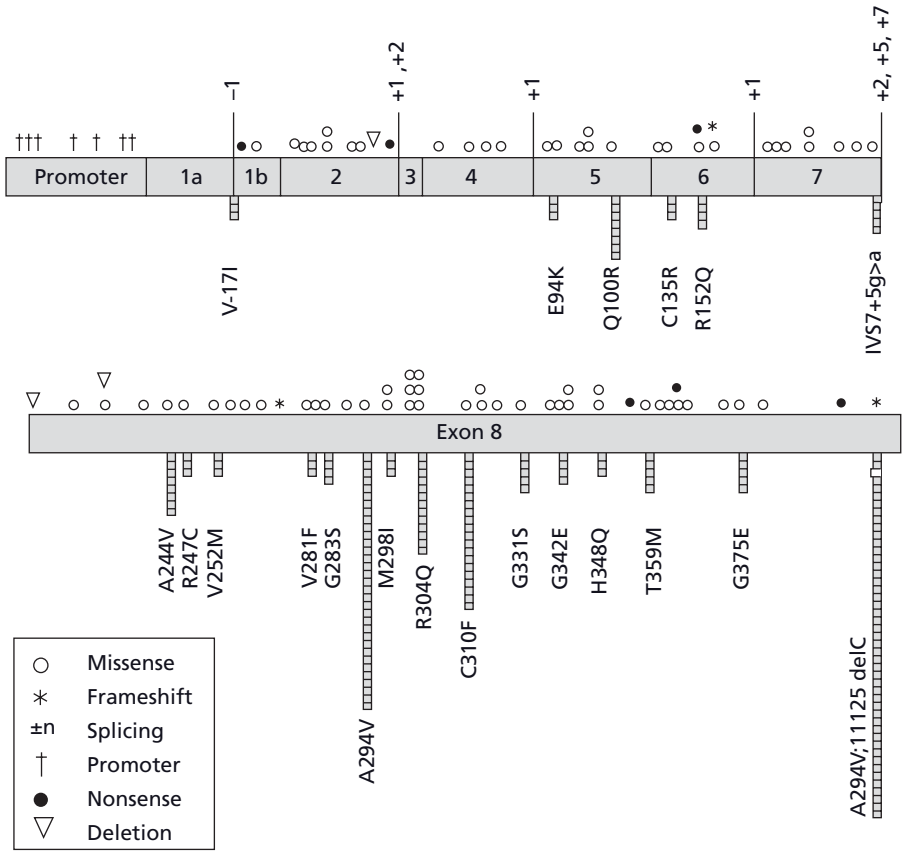

Figure 51.1 Mutational spectrum in FVII deficiency from the IRF7 database. Gray, horizontal bars denote promoter and cDNA segments. Splicing mutations are indicated through the position in the consensus splicing sequence. Mutations present in more than five alleles are reported by the bars; one square = two alleles [15].

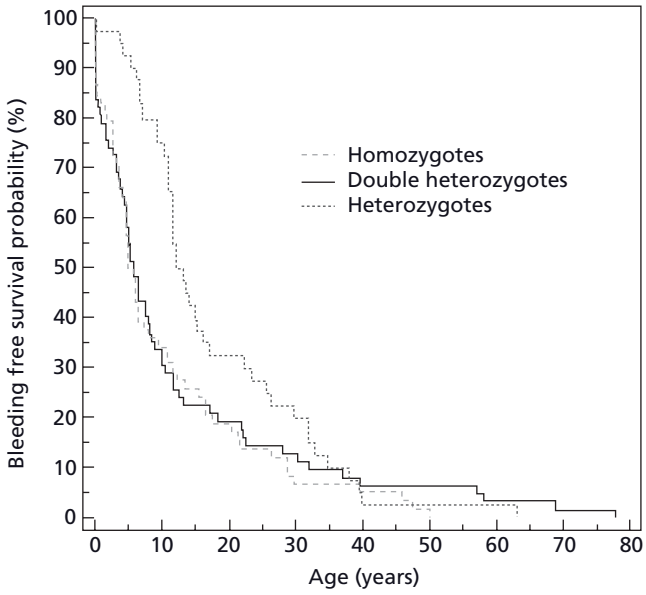

Figure 51.2 FVII deficiency: bleeding-free survival curves by zygosity state (IRF7 database).

Splicing-site changes are also well represented [27] and nonsense mutations and small deletions are rare, particularly in the homozygous condition. A frequent allele [13] bearing a double change, the missense Ala294Val mutation and a single nucleotide deletion, 11125delC, causing an elongated protein with additional 28 residues, occurs in an appreciable number of subjects, particularly in central Europe. Several mutations located in CpG sequences, known to be "hot spot" mutation sites, have been found in a number of patients coming from different countries. Virtually all of the moderate and severe patients are either homozygotes or double heterozygotes for mutations, and the clotting and clinical phenotypes of double heterozygotes and homozygotes are virtually indistinguishable [15].

The homozygous condition has a variable prevalence in different countries, and has been associated with consanguinity, caused by genetic isolates and/or ethnic customs. Patients homozygous for the same mutation do not always belong to the same class of severity, indicating phenotypic heterogeneity in the presence of identical *F7* gene mutations [15,27]. These observations, as well as the report of a number of symptomatic heterozygotes, point toward the existence of environmental factors and/or of genetic components [15,27,28] with the capacity of modifying the function of the clotting process and the expressivity of FVII deficiency. At any rate, in terms of age of disease presentation, homozygotes and double heterozygotes are indistinguishable (Figure 51.2).

Plasma levels of FVII and FVIIa in the general population are known to be affected by several polymorphisms in the *F7* gene [21,22], but their contribution to the clinical phenotype

in FVII deficiency seems to be minimal, if any [15]. A fine carrier detection approach, based on the clotting phenotype, has also been proposed [29].

Genetic analysis of exons, splicing, and promoter regions indicate causative mutations in the vast majority of patients [14,15], but in a few patients (<10%) no mutation is found after this type of screening. However, the extensive sequencing of the whole gene, which would corroborate these observations, has not been reported. This makes FVII deficiency caused by mutations in genes different from *F7* gene still an open question.

Prenatal diagnosis in severe FVII deficiency has been successfully performed by molecular genetic methods. The presence of repeated regions, and of polymorphic variation of repeats within the FVII genes, requires a careful design of polymerase chain reaction (PCR) primers [30].

Clinical manifestations and disease presentation

The most frequent symptoms [Table 51.1 and Figure 51.3 (grey bars)] indicate that in the majority of cases the disease is a mild one, mimicking the clinical picture of a platelet disorder, epistaxis, and easy bruising; gum bleeding being the most frequent type of hemorrhage. However, severe to very severe cases are not infrequent, characterized, as disease presentation, by central nervous system (CNS) and gastrointestinal (GI) bleeding and, later, when children start to crawl or walk, by hemarthrosis and muscle hematomas. Also, postoperative bleeding may occur, even though clotting tests may not help in predicting the bleeding risk [21].

Thromboses, particularly on the venous side, have been reported in 3–4% of patients with FVII deficiency, mainly following surgery and prolonged replacement treatments; however, "spontaneous" thrombotic episodes have also been reported [31].

Our group (International Registry on FVII Deficiency, IRF7) has consistently shown that age and type of the first symptom are variables which correlate strongly with the clinical severity

Table 51.1 Symptom distribution in the IRF7 database.

	Patients (n = 228)	
Symptoms	n	%
Epistaxis	190	83
Easy bruising	143	62
Gum bleeding	95	42
Muscle hematoma	57	21
Hemarthrosis	58	22
Gastrointestinal bleeding	44	14
Hematuria	26	12
Central nervous system bleeding	17	7
Postoperative bleeding	78	34

Only symptoms present in at least 5% of patients are reported. The prevalence of menorrhagia is reported in Table 51.2.

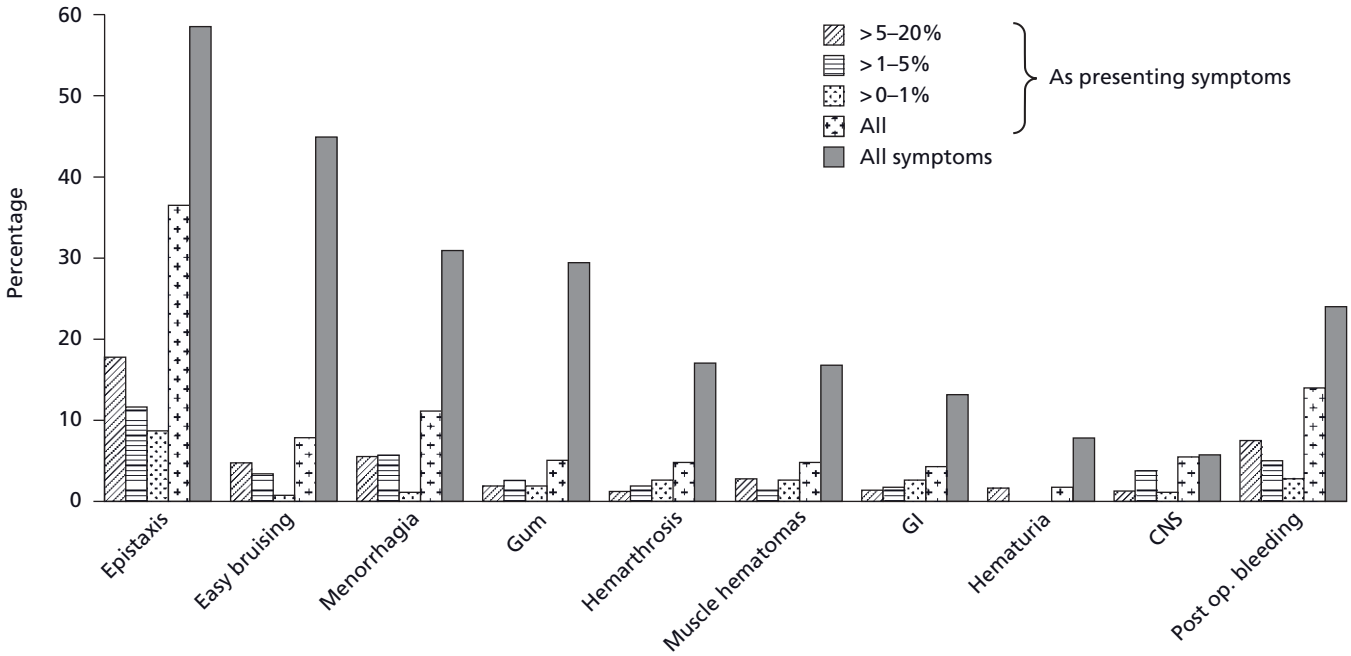

Figure 51.3 Grey bars denote the prevalence of each symptom in FVII deficiency (IRF7). The other bars refer to the "presenting symptoms" with prevalence referred to residual FVII levels or concerning the whole case report.

Table 51.2 Symptom distribution in FVII-deficient females (IRF7 data) (n = 174).

Symptoms	*n*	% patients
Epistaxis	98	56
Easy bruising	83	48
Gum bleeding	59	34
Muscle hematoma	28	16
Hemarthrosis	28	16
Gastrointestinal bleeding	24	14
Hematuria	9	5
Central nervous system bleeding	8	5
Thrombosis	5	3
Postoperative bleeding	40	30
Menorrhagia	100	63

The incidence of menorrhagia has been determined in females aged >10 and <50 years.

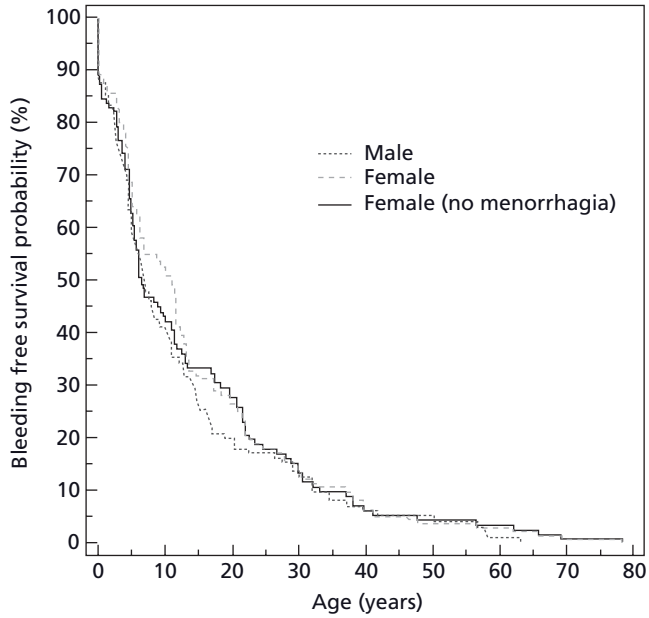

Figure 51.4 Bleeding-free survival analysis in FVII deficiency by gender (IRF7 database).

of the disease [15,32]. The main explanation for this is that these variables are not influenced by treatment or prophylaxis. This is clearly shown by (i) the bleeding-free survival curves where age of the first symptom is evaluated against the disease zygosity (Figure 51.2), (ii) the prevalence analysis of the different symptoms in relation to the residual FVII levels (<1%, 1–5%, and 5–20%) (Figure 51.3), and (iii) the fact that severe cases are characterized by an excessive prevalence of CNS and GI bleeding and joint and muscle bleeds as first symptom. CNS and GI bleedings were shown to occur mainly in babies or infants [15]. In homozygous and double heterozygous patients the age at which the first bleeding episode occurs is virtually indistinguishable (Figure 51.2).

As in the other autosomally inherited congenital bleeding disorders, menorrhagia is a very frequent type of bleeding in women with FVII deficiency [15,33,34] (Table 51.2), accounting for two-thirds of the bleeding incidence among women aged 10–50 years with a peak prevalence in teenagers. Menorrhagia may be associated with other gynecologic (i.e., hemoperitoneum related to ovarian cysts, metrorrhagia due to uterine fibromatosis) or obstetric (postpartum hemorrhage) problems and frequently leads to a profound iron depletion and anemia.

Other than gynecologic hemorrhage, women with FVII deficiency do not display a bleeding tendency, which is different from males (Tables 51.1 and 51.2). Further, the analysis of gender-comparative bleeding-free survival curves is characterized by very similar median bleeding-free survivals and curve shapes, independent of the presence of menorrhagia (Figure 51.4).

Diagnosis/clotting phenotype analysis

The diagnosis of FVII deficiency is easy and based on the discordance between a prolonged prothrombin time (PT) and a normal activated partial thromboplastin time (aPTT). PT-INR prolongation may be from moderate (1.5–1.8) to markedly elevated (>2.0), depending on FVII coagulant activity (FVIIc) levels. FVIIc is the confirmatory test for diagnosis [36]. However, particular laboratory rigor is required for evaluating levels around or below 1%. Factor VII antigen can be quantitatively assayed with polyclonal antibodies to FVII by an enzyme-linked immunosorbent assay (ELISA) (Stago) [35] or by using an amydolytic method [12]; antigen assay is useful to pick up the dysfunctional forms of FVII deficiency, characterized by a discrepancy between clotting and antigen levels. FVIIAg assay has a sensitivity similar to that of the clotting assay. FVIIa determination can be performed by clotting-based (Stago) or ELISA; however, this test is not routinely performed.

It is worth noting that PT-INR and FVIIc assay, when performed with recombinant-TF preparations, are very sensitive to FVIIa. The quality of deficient plasmas is very important for measuring low levels with accuracy as small amounts of residual FVII are present in most of these substrates. More sensitive activity methods are those based on the thrombin generation assays especially when fluorogenic substrates for the detection of thrombin are used; these methods are made more sensitive by using highly diluted TF to trigger the reaction [36].

Although the diagnosis of FVII deficiency is straightforward, there may be discordancy between onset of the bleeding symptoms and a formal laboratory diagnosis of FVII deficiency. Figure 51.5 compares age at first symptom and age at laboratory diagnosis in 214 symptomatic patients: these patients became symptomatic at a median age of 7.7 years,

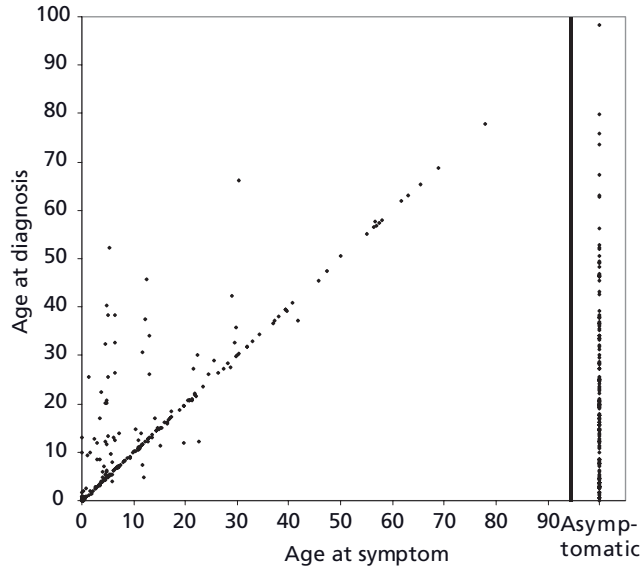

Figure 51.5 Scatter plot comparing age at the first symptom with age at laboratory diagnosis. On the right side of the graph, detail concerning age at diagnosis for asymptomatic individuals with a FVII deficiency.

whereas formal diagnosis of FVII deficiency was made at a median age of 11.8 years. Only 51.4% of subjects with FVII deficiency were diagnosed within 6 months after the first symptom.

At present, FVII activity assay is used to monitor replacement therapy of patients deficient in FVII. Diagnosis can be made using either zymogen and FVIIa assays, although the latter is more sensitive. For routine monitoring at the bedside the PT-INR may suffice as its normalization roughly correlates with the attainment of hemostatic levels.

Inhibitors to FVII are a rare event (possibly <1% of the treated cases) and little is known about their kinetic features.

Isolated FVII deficiency can easily be differentiated from the so-called FMFD (familial multiple factor deficiency) type III as in the latter the aPTT is also prolonged, the levels of the other vitamin K-dependent proteins (also proteins C and S) are depressed, and, finally, because this condition is, at least partially, responsive to vitamin K.

FVII deficiency has been found to be associated with hepatic congenital enzymic defects, such as the Dubin–Johnson and Gilbert syndromes [37,38].

Treatment

FVII is a rare protein (350–450 ng per mL of plasma) with a very short half-life, especially in children [39,40]. In fact, we should think in terms of FVIIa levels, the active portion of the total FVII mass; however, this serine protease, because of the extremely low levels, is difficult to assay in FVII deficiency.

Table 51.3 Treatment materials for congenital FVII deficiency.

Materials	Potency (IU/mL)[a]	Advantages	Disadvantages
Fresh-frozen plasma	1	Easily available, low cost	Limited effectiveness; risk of circulatory overload; risk of virus transmission
Virally attenuated fresh-frozen plasma	1	Same	Limited effectiveness; risk of circulatory overload
Prothrombin complex concentrates (four factors) (PCCs)	5–10	Suitable for surgery; virally attenuated	Other vitamin K-dependent factors present in concentrations higher than FVII, possibly activated; risk of thrombosis; not easily available
Plasma-derived FVII concentrate[b]	20–30	Suitable for surgery; virally attenuated; effective	Other vitamin K-dependent factors present in high concentrations, possibly activated; risk of thrombosis
Recombinant, activated FVII (rFVIIa)[c]	>25 000	Very effective for all the indications at relatively low doses; widely available; no risk of viral transmission	—

[a] 1 IU corresponds to the amount of FVII present in 1 ml of fresh-frozen plasma.
[b] Provertin UM TIM3, Baxter, Deerfield, IL, USA.
[c] Novoseven™, Novonordisk, Bagsvaerd, Denmark.

The low plasma levels of FVII and its properties similar to those of the other vitamin K-dependent clotting factors makes the preparation of high-purity concentrates from plasma difficult; its presence as a trace protein makes FFP unsuitable for prolonged treatments.

For FVII-deficient patients there are a number of treatment options which may result in a very effective replacement therapy. Available treatment materials, the potency of these products relative to plasma, and their respective advantages and drawbacks are shown in Table 51.3.

Fresh-frozen plasma is still used in developing countries, the most important adverse outcomes from its use being blood volume overload and a relatively high risk of transmitting blood-borne viruses. Plasma-derived FVII concentrates are

essentially PCCs with a significantly higher content of FVII relative to the other vitamin K-dependent proteins. These are effective for any therapeutic requirement. Our group described, in a retrospective study, a number of cases with post-treatment thrombosis mostly concerning the venous site [32]. Thrombotic episodes were not related to any known thrombophilia.

Recombinant FVIIa (rFVIIa, Novoseven®) is today considered the first-line product for replacement therapy in FVII deficiency. As FVIIa is the protein to replace (the zymogen has very little enzymic activity) and considering the very high potency of Novoseven, the latter can be used at a low dose (15–30 μg/kg b.w.) [41,42] in comparison with refractory hemophilia. For the severe bleeds, and the surgical interventions, multiple administration schedules should be adopted two or three times daily [42].

Prophylaxis has been a debated issue in FVII deficiency, especially because of the very brief persistence of infused FVII in the bloodstream. Recent anecdotal clinical observations have shown that secondary prophylaxis can be performed with FFP infusions, in spite of the very low concentrations of FVII. In addition, *ex vivo* studies showed that infused FVIIa disappears quickly from the circulation but persists extravascularly bound to pericytes [43]. These observations would support the feasibility of prophylaxis in FVII deficiency but trials are needed to assess the optimal schedule. At any rate, the main target for prophylaxis are the newborns who have had early and severe (CNS, GI) bleeds.

Little evidence is also available for the short prophylaxis courses aimed at the prevention of bleeding during and after surgical interventions: a wide array of dosages and schedules have been employed [42]. In some cases, surgery has been performed without any replacement therapy, namely in patients with negative bleeding history [44], but these patients were not uniform in terms of genotype nor clotting phenotype so it is difficult to identify those who are not likely to bleed.

For both bleeding prevention and treatment of spontaneous bleeds, plasma-derived FVII concentrates and recombinant FVIIa seem to be equally effective but no formal comparative studies have been published. Both preparations are suitable for home care. As for the single standard dose capable of inducing hemostatic levels, it ranges from 15 to 30 μg/kg b.w. for recombinant FVIIa and from 10 to 30 IU/kg b.w. for the pdFVII preparations (unpublished data from the STER registry). Considering rFVIIa, the average dose capable of normalizing the PT-INR in severely affected patients was found to be 20 μg/kg [41].

As regards the kinetics of infused FVII, scanty data are available, which confirm the very short half-life, especially in children [39,40].

Treatment complications, if one rules out the transmission of blood-borne viruses (very rare today), are confined to the occurrence of thrombosis (rare complication of replacement treatment, sometimes spontaneous) [31] or the rare appearance of inhibitors to FVII [45].

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References

1 Rapaport SI, Rao LV. The tissue factor pathway: how it has become a "prima ballerina." *Thromb Haemost* 1995; **74**: 7–17.
2 Morissey JH, Mutch N. Tissue factor structure and function. In: Colman RW, Marder VJ, Clowes AW, George JN, Goldhaber SZ. *Hemostasis and Thrombosis. Basic Principles and Clinical Practice*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, 2006: 91.
3 Colman RW, Clowes AW, George JN, *et al.* Overview of hemostasis. In: Colman RW, Marder VJ, Clowes AW, George JN, Goldhaber SZ. *Hemostasis and Thrombosis. Basic Principles and Clinical Practice*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, 2006: 3.
4 Bock SC. Antithrombin III and heparin cofactor II. In: Colman RW, Marder VJ, Clowes AW, George JN, Goldhaber SZ. *Hemostasis and Thrombosis. Basic Principles and Clinical Practice*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, 2006: 235.
5 Rosen ED, Xu H, Liang Z, Martin JA, Suckow M, Castellino FJ. Generation of genetically-altered mice producing very low levels of coagulation Factor VII. *Thromb Haemost* 2005; **94**: 493–7.
6 Mannucci PM, Duga S, Peyvandi F. Recessively inherited coagulation disorders. *Blood* 2004; **104**: 1243–52.
7 Perry DJ. Factor VII deficiency. *Br J Haematol* 2002; **118**: 689–700.
8 Roberts HR, Escobar MA. Less common congenital disorders of hemostasis. In: Kitchens CS, Alving BM, Kessler CM (eds.) *Consultative Hemostasis and Thrombosis*. Philadelphia: WB Saunders, 2002: 57.
9 Roberts HR, Escobar MA. Inherited disorders of prothrombin conversion. In: Colman RW, Marder VJ, Clowes AW, George JN, Goldhaber SZ. *Hemostasis and Thrombosis. Basic Principles and Clinical Practice*, 5th edn. Philadelphia: Lippincott Williams & Wilkins, 2006: 923.
10 Alexander B, Goldstein R, Landwehr G, Cook CD. Congenital SPCA deficiency: a hitherto unrecognized coagulation defect with hemorrhage rectified by serum and serum fractions. *J Clin Invest* 1951; **30**: 596–603.
11 Mariani G, Mazzucconi MG. Factor VII congenital deficiency. Clinical picture and classification of the variants. In: Written Symposium of FVII. *Haemostasis* 1983; **13**: 169–78.
12 Triplett DA, Brandt JT, Batard MA, Dixon JL, Fair DS. Hereditary factor VII deficiency: heterogeneity defined by combined functional and immunochemical analysis. *Blood* 1985; **66**: 1284–7.
13 Herrmann FH, Wulff K, Herrmann FH, *et al.* Molecular biology and clinical manifestations of hereditary FVII deficiency. *Sem Thromb Hemost* 2000; **26**: 393–400.
14 McVey JH, Boswell E, Mumford AD, Kembakk-Cook G, Tuddenham EGD. Factor VII deficiency and the FVII mutation database. *Hum Mutat* 2001; **17**: 3–17.
15 Mariani G, Herrmann FH, Dolce A, *et al.*, for the International Factor VII Deficiency Study Group. Clinical phenotypes and factor

VII genotype in congenital factor VII deficiency. *Thromb Haemost* 2005; **93**: 481–7.
16 Millar DS, Kemball-Cook G, Mc Vey JH, *et al.* Molecular analysis of the genotype–phenotype relationship in factor VII deficiency. *Hum Genet* 2000; **107**: 327–42.
17 Bernardi F, Castaman G, Pinotti M, *et al.* Mutation pattern in clinically asymptomatic coagulation factor VII deficiency. *Hum Mutat* 1996; **8**: 108–15.
18 Peyvandi F, Jenkins PV, Mannucci PM, *et al.* Molecular characterisation and three-dimensional structural analysis of mutations in 21 unrelated families with inherited factor VII deficiency. *Thromb Haemost* 2000; **84**: 250–7.
19 Herrmann FH, Wulff K, Auberger K, *et al.* Molecular biology and clinical manifestation of hereditary factor VII deficiency. *Semin Thromb Hemost* 2000; **26**: 393–400.
20 Wulff K, Herrmann FH. Twenty-two novel mutations of the factor VII gene in factor VII deficiency. *Hum Mutat* 2000; **15**: 489–96.
21 Giansily-Blaizot M, Verdier R, Biron-Adréani C, *et al.* Study group of FVII deficiency. Analysis of biological phenotypes from 42 patients with inherited factor VII deficiency: Can biological tests predict the bleeding risk? *Haematologica* 2004; **89**: 704–9.
22 Green F, Kelleher C, Wilkes H, Temple A, Meade T, Humphries S. A common genetic polymorphism associated with lower coagulation factor VII levels in healthy individuals. *Arterioscler Thromb* 1991; **11**: 540–6.
23 Bernardi F, Marchetti G, Pinotti M, *et al.* Factor VII gene polymorphisms contribute to one third of the factor VII level variation in plasma. *Arterioscler Thromb Vasc Biol* 1996; **16**: 72–6.
24 Hagen FS, Gray CL, O'Hara P, *et al.* Characterization of a cDNA coding for human factor VII. *Proc Natl Acad Sci USA* 1987; **84**: 5158–62.
25 O'Hara PJ, Grant FJ, Haldeman BA, *et al.* Nucleotide sequence of the gene coding for human factor VII, a vitamin K-dependent protein participating in blood coagulation. *Proc Natl Acad Sci USA* 1986; **83**: 2412–16.
26 Pinotti M, Rizzotto L, Pinton P, *et al.* Intracellular readthrough of nonsense mutations by aminoglycosides in coagulation factor VII. *J Thromb Haemost* 2006; **4**: 1308–14.
27 Castoldi E, Govers-Riemslag JW, Pinotti M, *et al.* Coinheritance of Factor V (FV) Leiden enhances thrombin formation and is associated with a mild bleeding phenotype in patients homozygous for the FVII 9726+5G>A (FVII Lazio) mutation. *Blood* 2003; **102**: 4014–20.
28 Kario K, Narita N, Matsuo T, *et al.* Genetic determinants of plasma FVII in the Japanese. *Thromb Haemost* 1995; **73**: 617–22.
29 Mariani G, Hermans J, Orlando M, *et al.* Carrier detection in FVII congenital deficiency. *Br J Haematol* 1985; **61**: 687–94.
30 Giansily-Blaizot M, Thorel D, Khau Van Kien P, *et al.* Characterisation of a large complex intragenic re-arrangement in the FVII gene (*F7*) avoiding misdiagnosis in inherited factor VII deficiency. *Br J Haematol* 2007; **138**: 359–65.
31 Mariani G, Herrmann FH, Schulman S, *et al.* International Factor VII Deficiency Study Group. Thrombosis in inherited factor VII deficiency. *J Thromb Haemost* 2003; **1**: 2153–8.
32 Bernardi F, Dolce A, Pinotti M, *et al.* for the International Factor VII Deficiency Study Group. Major differences in bleeding symptoms between factor VII deficiency and haemophilia B. *J Thromb Haemost* 2009; **7**: 774–9.
33 Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. Frequency of inherited bleeding disorders in women with menorrhagia. *Lancet* 1998; **351**: 485–9.
34 Lak M, Peyvandi F, Mannucci PM. Clinical manifestations and complications of childbirth and replacement therapy in 385 Iraq patients with type 3 von Willebrand disease. *Br J Haematol* 2000; **111**: 1236–9.
35 Mariani G, Liberti G, D'Angelo T, LoCoco L. Factor VII activity and antigen. In: Jespersen J, Bertina RM, Haverkate F (eds)., *Laboratory Techniques in Thrombosis. A Manual*. Dordrecht: Kluwer Academic Publishers, 1999: 99–106.
36 Pinotti M, Etro D, Bindini D, *et al.* Residual factor VII activity and different hemorrhagic phenotypes in CRM(+) factor VII deficiencies (Gly331Ser and Gly283Ser). *Blood* 2002; **99**: 1495–7.
37 Levanon M, Rimon S, Shani M, *et al.* Active and inactive factor VII in Dubin–Johnson syndrome with factor VII deficiency, hereditary factor VII deficiency and coumarin administration. *Br J Haematol* 1972; **23**: 669.
38 Seligshon S, Shani M, Rabot B. Gilbert syndrome and factor VII deficiency. *Lancet* 1979; **i**: 1398.
39 Mariani G, Mannucci PM, Mazzucconi MG, Capitanio A. Treatment of congenital factor VII deficiency with a new concentrate. *Thromb Haemost* 1978; **39**: 675–9.
40 Berrettini M, Mariani G, Schiavoni M, *et al.* Pharmacokinetic evaluation of recombinant, activated factor VII in patients with inherited factor VII deficiency. *Haematologica* 2001; **86**: 640–5.
41 Mariani G, Testa MG, Di Paolantonio T, *et al.* The use of recombinant, activated FVII in the treatment of congenital factor VII deficiencies. *Vox Sang* 1999; **77**: 131–6.
42 Mariani G, Konkle BA, Ingerslev J. Congenital factor VII deficiency: therapy with recombinant activated factor VII—a critical appraisal. *Haemophilia* 2006; **12**: 19–27.
43 Hoffman M, Colina CM, McDonald AG, Arepally GM, Pedersen L, Monroe DM. Tissue factor around dermal vessels has bound factor VII in the absence of injury. *J Thromb Haemost* 2007; **5**: 1403–8.
44 Giansily-Blaizot M, Biron-Andreani C, Aguilar-Martinez P, *et al.* Inherited factor VII deficiency and surgery: clinical data are the best criteria to predict the risk of bleeding. *Br J Haematol* 2002; **117**: 172–5.
45 Ingerslev J, Christiansen K, Sørensen B; International Registry on Factor VII Deficiency (IRF7) Steering Committee. Inhibitor to factor VII in severe factor VII deficiency: detection and course of the inhibitory response. *J Thromb Haemost* 2005; **3**: 799–800.

52 Factor X and factor X deficiency

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Introduction

Factor X (synonyms: autoprothrombin III, Stuart–Prower factor) is one of the vitamin K-dependent clotting factors and occupies a central position in the coagulation cascade at the point of convergence of the so-called "intrinsic" and "extrinsic" pathways. Factor X was identified in the early 1950s by two groups who independently reported a novel clotting factor, deficient in patients with a hemorrhagic tendency and which closely resembled factor VII [1,2]. The deficient clotting factor in these individuals was termed "Stuart–Prower" factor after the index patients. In 1954, Duckert and colleagues reported a factor deficient in patients receiving coumarin anticoagulants that was distinct from factor VII and IX and which they called factor X before the official Roman numeral nomenclature was established in 1962 [3,4].

F10 gene

The gene for factor X (*F10*) maps to the long arm of chromosome 13 at 13q34–ter approximately 28 kb downstream of the *F7* gene [5]. The gene consists of eight exons spread over ~27 kb of genomic sequence (Figure 52.1) and shares significant homology with the other vitamin K-dependent clotting factors both in organization and structure [6–8]. Each of the eight exons of the *F10* gene encode specific domains of the factor X protein:

- exon I encodes the signal peptide;
- exon II encodes the propeptide and γ-carboxyglutamic acid-rich (Gla) domain;
- exon III encodes a short linking segment of aromatic amino acids residues termed the "aromatic stack;"
- exons IV and V encode regions homologous to epidermal growth factor (EGF);
- exon VI encodes the activation peptide at the amino-terminus of the heavy chain; and
- exons VII and VIII encode the active serine protease domain containing the catalytic triad His236, Asp228, and Ser379.

The factor X cDNA consists of 120 bp coding for the 40-amino-acid pre-pro-leader sequence, 1344 bp encoding the 488 amino acids of the mature protein and a 3′ untranslated region of 10 bp preceding the poly(A) tail (Figure 52.1) [9].

Factor X protein: structure and function

Factor X is synthesized by the liver and secreted into the plasma as an inactive zymogen where it circulates as a two-chain molecule with a concentration of ~8–10 μg/mL. Factor X is synthesized with a 40-residue pre-pro-sequence which contains the hydrophobic signal sequence that targets the protein for secretion (residues –37 to –22) and which is cleaved prior to secretion of the mature protein into the plasma. Extensive post-translational modifications (glycosylation, γ-carboxylation, and β-hydroxylation) of factor X occur before it is functionally active.

The first 39 residues of the light chain of factor X contain 11 glutamic acid residues which are modified by a vitamin K-dependent γ-carboxylation step to form γ-carboxyglutamic acid (Gla) residues. Ten of these residues are encoded by exon II (the "Gla" domain) and the eleventh by exon III. Gla residues mediate conformational changes in factor X that allow Ca^{2+}-dependent binding to negatively charged phospholipid membranes.

In its two-chain form, mature factor X consists of a light chain of 139 amino acids and a heavy chain of 346 residues. The two chains are connected by an Arg–Lys–Arg (RKR) tripeptide and by a disulfide bond between residues Cys89 and Cys124 (Figure 52.1). The heavy chain of factor X contains the activation peptide (residues 143–195), which is cleaved when factor X is activated. The heavy chain also contains the triad of amino acids—His236, Asp282, and Ser379—which constitute the catalytic site.

No crystal structure for native factor X exists, although a number of partial structures have been solved [10–13] and subsequently used to model a number of the factor X mutations [13].

The role of factor X

Physiologically, factor X is activated by factor IXa or by factor VIIa although *in vitro* factor X can also be activated by Russell viper venom, a metalloproteinase isolated from the venom of the snake *Vipera russelli*. Factor X is converted to factor Xa

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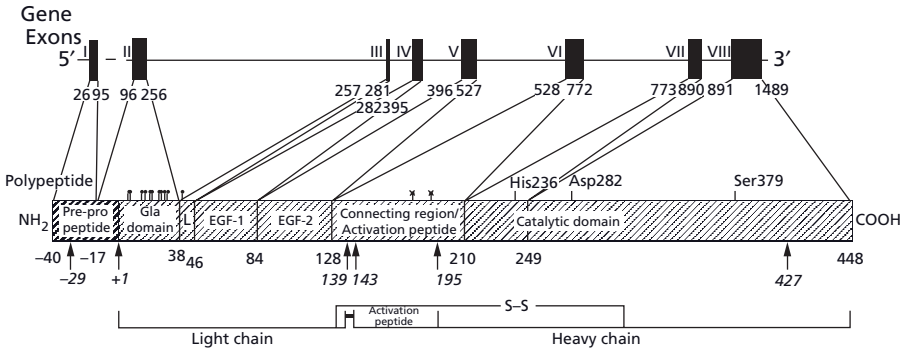

Figure 52.1 The human factor X gene (*F10*) and its encoded polypeptide. The upper part of the figure represents the *F10* gene with the eight exons shown by filled boxes. The positions of the first and last nucleotides of each exon are shown (nucleotides are numbered according to Leytus *et al.* [47]). The lower part of the figure shows the polypeptide structure and the various functional domains encoded by specific exons. Darker hatching indicates the pre-propeptide and lighter hatching the mature protein. Codons initiating each exon are shown (residue +1 is the first amino acid of the mature protein). Residue –29 indicates the site of probable cleavage by the signal peptidase. The light chain is encoded by residues +1 to 139 and the heavy chain by residues 143–448. The connecting tripeptide (RKR) is located between residues 140 and 142 and the activation peptide resides within the heavy chain at residues 143–195. Activation of factor X occurs through cleavage at Arg194/Ile195. Residue 427 shows the site of cleavage of factor X that generates factor Xaβ. His236, Asp282, and Ser379 are the residues which constitute the catalytic triad. (↑) the position of the 11 Gla residues; (↟) the position of the two glycosylation sites.

by cleavage of the Arg194–Ile195 peptide bond located in the heavy chain. Cleavage releases a 52-residue peptide—the factor X activation peptide, which has been used a marker of factor X activation [14].

Activation of the coagulation cascade occurs following exposure of tissue factor (TF) to plasma. The formation of a complex comprising tissue factor, factor VIIa, and calcium ions in the presence of an appropriate phospholipid membrane activates factor X to Xa and factor IX to IXa. A variety of cell types including endothelial cells, fibroblasts, monocytes, macrophages, and tumor cells appear to be capable of providing a suitable phospholipid surface for activation. The presence of all the constituents results in a 10-fold decrease in K_m for the reaction and a 1000-fold increase in k_{cat} accelerating the generation of factor Xa by at least 10 000-fold relative to rates observed for factor VIIa and Ca^{2+} alone.

Activation of factor X via the extrinsic pathway is dependent upon the interaction of factor IXa, factor VIIIa, Ca^{2+}, and a phospholipid surface—platelets and/or endothelial cells. Factor VIIIa serves as a cofactor in the activation of factor X accelerating the maximal velocity of the reaction approximately 200 000-fold, whereas phospholipid has been shown to significantly decrease the K_m of factor IXa for factor X by some 5000-fold.

Factor Xa is the major physiologic activator of prothrombin but its enzymatic activity is accelerated some 280 000-fold in the presence of factor Va, Ca^{2+} ions, and a suitable negatively charged phospholipid membrane. The phospholipid membrane provides a surface which increases the local concentration of factor Va, Xa, and prothrombin, although factor Va also appears to increase the catalytic efficiency of factor Xa.

The principal regulators of factor X activity (FXa) are antithrombin and tissue factor pathway inhibitor (TFPI). However, protein Z (PZ), a plasma vitamin K-dependent protein, functions as a cofactor to enhance the inhibition of FXa by the serpin, protein Z-dependent protease inhibitor (ZPI) [15]. Factor Xa is inhibited by antithrombin to form a stable inactive complex which is rapidly removed from the circulation by the liver. The inhibitory activity of antithrombin is accelerated by various sulfated glycosaminoglycans. TFPI is the major inhibitor of the extrinsic pathway and rapidly forms a 1:1 complex with factor Xa which then binds to the tissue factor–factor VIIa complex to form a quaternary complex [16]. The quaternary Xa–TFPI–TF–VIIa complex lacks any VIIa–TF catalytic activity.

Factor X deficiency

Diagnosis

The diagnosis of factor X deficiency is suspected following the finding of a prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT) which corrects (unless an inhibitor is present) in a 50:50 mix with normal plasma. The diagnosis of factor X deficiency is confirmed by measuring plasma factor X levels. Factor X can be measured either immunologically or functionally. Numerous methods exist to measure factor X antigen in plasma including electroimmunoassay, antibody neutralization, immunodiffusion, radioimmunoassay, laser nephelometry, and enzyme-linked immunosorbent assay (ELISA). Factor X antigen is reduced in some cases of factor X deficiency in which there is a total

reduction in the levels of circulating factor X but it may be entirely normal in patients with a dysfunctional factor X molecule. Factor X antigen levels are reduced to approximately 50% of normal in patients on coumarin therapy, although factor X activity is lower [17]. The reduction in factor X antigenic levels in such patients may reflect an increased catabolism of the acarboxylated form or to a reduced secretion by the hepatocyte.

The most widely used functional factor X assay is the one-stage factor X assay which employs factor X-deficient plasma as a substrate to which dilutions of normal or test plasma are added and the correction in clotting times (either the PT or aPTT) is compared. Russell viper venom (RVV) can also activate factor X and by using factor X-deficient substrate plasma, an assay can be devised that is specific for factor X. As with the PT and aPTT-based assays for factor X, some variants have been reported in which the RVV assay of factor X is normal [18].

A chromogenic factor X assay offers an accurate and reliable alternative to clotting assays, although the nonspecific nature of the substrate may give rise to spuriously high results in some patients with factor X deficiency (Perry DJ, Owens D, Riddell A, unpublished results). A number of factor X variants have been described with varying effects upon factor X levels depending upon the assay method employed [19–21]. For these reasons an approach to suspected factor X deficiency may require a number of different assays [22].

Factor X levels are low at birth and age- or gestation-related ranges for factor X must be used if a deficiency is suspected [23]. The diagnosis of mild factor X deficiency may be especially difficult in premature or young neonates when vitamin K deficiency may complicate assessment. Reassessment after vitamin K replacement may be necessary. It is important to exclude vitamin K deficiency or other acquired causes of a clotting disorder before the diagnosis of factor X deficiency is made. In some cases, family studies may help in establishing the diagnosis of factor X deficiency.

Clinical features

Factor X deficiency is inherited as an autosomal recessive disorder. It is among the rarest of the inherited coagulation disorders with an estimated prevalence in the UK of 1 in 500 000, but in its heterozygous form it is more common, with an estimated frequency of ~1 in 500, although such individuals are usually asymptomatic. Rarely, heterozygotes can exhibit bleeding symptoms [24]. This may be because of either the insufficient enzymatic activity by the normal factor X or an inhibition of a reaction step in the coagulation pathway by the mutant gene product. Because factor Xa is part of the prothrombinase complex, a dysfunctional factor Xa molecule may compete with the normal factor Xa for factor Va binding sites. The net effect would be to decrease the formation of an active prothrombinase complex.

In contrast to hemophilia A and B, in which the risk of bleeding increases as the factor VIII or IX level falls, factor X deficiency shows a variable bleeding tendency with a poor correlation between absolute factor levels and bleeding (Figure 52.2). Less severely affected patients may bleed only after a challenge to the hemostatic system (e.g., trauma or surgery).

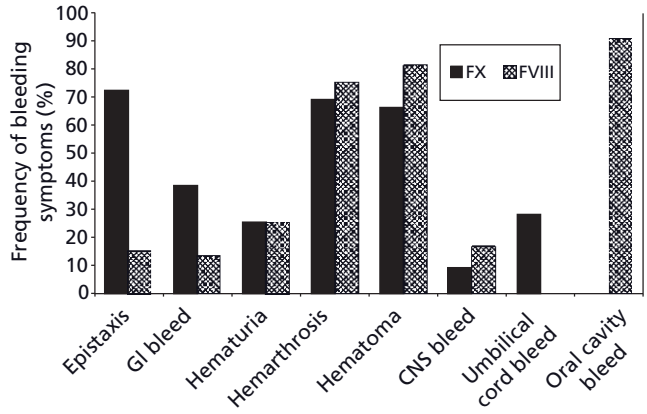

Figure 52.2 Prevalence of bleeding symptoms in 150 patients with hemophilia A (FVIII) matched for age and severity with patients with factor X deficiency.

Patients with factor X deficiency may present at any age and severely affected individuals (FX:C < 1 U/dL) can present in the neonatal period with umbilical-stump bleeding. The most frequent symptom in factor X deficiency is epistaxis and this is seen with all severities of deficiency. Other mucosal-type bleeding is less frequent and occurs mainly in patients with severe deficiencies. Menorrhagia occurs in half the women of reproductive age. Hemarthroses, severe postoperative hemorrhage and central nervous system hemorrhage have been reported. Recurrent hemarthroses may result in severe arthropathy. Moderately affected patients (FX:C 1–5 U/dL) may bleed only after hemostatic challenge, e.g., trauma or surgery. Mild factor X deficiency (FX:C 6–10 U/dL) may be identified incidentally during routine screening or family studies. Patients who are only mildly affected may experience easy bruising or menorrhagia.

In a study of 102 patients from central Europe and Latin America, the bleeding symptoms in 42 symptomatic individuals (26 homozygotes, seven compound heterozygotes, and nine heterozygotes) comprised easy bruising (55%), hematomas (43%), epistaxes (36%), hemarthroses (33%), intracranial hemorrhage (ICH; 21%), and gastrointestinal (GI) hemorrhage (12%). The most severe clinical symptoms (ICH, GI bleeding, and hemarthroses) occurred in cases with FX:C < 2%. In this study specific mutations appeared to be correlated with a particular bleeding phenotype, e.g., ICH seemed to be associated with the *F10* mutation Gly380Arg, and possibly with the mutations IVS7–1G>A and Tyr163delAT.

The thrombogram has been used to investigate the relation between factor X levels in an attempt to establish at what level of factor X normal thrombin generation is achieved [25]. In factor X deficiency, half-normal endogenous thrombin

potential (EPT) was seen at a factor X concentration of 5%. Ten individuals with factor X deficiency (factor X levels 1–50% of normal) were studied and when functional factor X activity was below 10% the parameters of the thrombogram—lag-time and peak height—were markedly abnormal. The endogenous thrombin potential was similar irrespective of the method of activation of factor X (extrinsic pathway or intrinsic pathway). In patients with a factor X activity between 10% and 50% only the lag-time of thrombogram and the peak height were abnormal, but the ETP remained within normal limits. These patients had no bleeding even after trauma and this work suggests that the threshold range of factor X required to obtain normal thrombin generation is approximately 10% of normal.

Classification of factor X deficiency

Classical hereditary factor X deficiency (CRM^-) is characterized by a prolonged PT, prolonged aPTT, prolonged RVV time, and deficiencies in both factor X activity and antigen. In contrast, CRM^+ disease has been described and in such cases factor X antigen is normal or near normal but the PT, aPTT, RVV, and chromogenic factor X assays show variable activity.

A tentative classification for factor X deficiency based upon the results of various functional (PT, aPTT, and RVV time-based assays) and immunologic assays was proposed some years ago although is not in common usage [26]. However, it is clear that defects that affect only one assay are associated with a milder phenotype and that factor X deficiency associated with low levels in multiple assays are associated with a more severe phenotype [22].

Molecular basis of factor X deficiency

Factor X mutations are thought to be rare because of the central role of factor X in the coagulation cascade. Factor X knockout mice show embryonic or perinatal lethality and this is consistent with the hypothesis that a complete absence of factor X is a lethal disorder [27]. However, mice with factor X activity levels of 1–3% show complete rescue of lethality [28].

The earliest reported molecular abnormality affecting the *F10* gene was reported by Scambler and Williamson who described a female monosomic for 13q34 and who was found to be deficient in factors VII and X [29]. Interestingly, her brother was trisomic for 13q34 and had elevated levels of these factors. Chromosomal deletions affecting both the *F10* and the *F7* genes, which are positioned close to each other, form part of the 13q– syndrome [30].

Most mutations reported in factor X deficiency are single point mutations resulting in amino acid substitutions (missense mutations) and affecting primarily exon 8, the largest exon in the *F10* and which encodes the catalytic domain. Several cases of factor X deficiency have been reported in which there is also a deficiency of the other vitamin K-dependent clotting factors. Hereditary combined vitamin K-dependent coagulation factor deficiency is an autosomal recessive bleeding disorder associated with defects in either the γ-carboxylase, which carboxylates vitamin K-dependent proteins to render them active, or the vitamin K epoxide reductase (VKORC1), which supplies the reduced vitamin K cofactor required for carboxylation.

Acquired factor X deficiency

An acquired rather than an inherited deficiency of factor X is seen in a number of diverse disorders.

1 *Liver disease/oral anticoagulants/vitamin K deficiency.* The differentiation of inherited factor X deficiency from acquired deficiencies should include consideration of liver disease and vitamin K deficiency (malabsorption, warfarin, and other oral anticoagulants), although in such cases there are commonly deficiencies of the other vitamin K-dependent or hepatic-derived clotting factors, e.g., factor V.

2 *Amyloidosis.* An association between amyloidosis and acquired factor X deficiency was reported by Korsan-Bengsten in 1962 [31] and subsequently by others. In general, there is only a modest reduction in factor X antigen but a marked reduction in factor X activity. Removal of endogenous factor X from the plasma of these patients, as well as exogenous factor X, appears to be mediated via the amyloid fibrils which are deposited throughout the vasculature. Factor X has been shown to bind directly to amyloid fibrils. Treatment of the amyloidosis has limited benefit but may result in some improvement in factor X levels. Splenectomy has been reported to be useful probably because the spleen acts a large reservoir of amyloid material. Exogenously administered factor X is rapidly cleared from the circulation and is, therefore, of little, if any, benefit.

3 *Miscellaneous.* Factor X deficiency has been reported in association with a number of other disorders, including myeloma (without amyloidosis), following exposure to the fungicide methylbromide, in association with various tumors and in acute myeloid leukemia treated with amsacrine. Two cases of acquired factor X deficiency in patients with leprosy and a single case in association with a *Mycoplasma pneumoniae* chest infection have been documented.

Treatment of factor X deficiency

Inherited factor X is a rare disorder and there are no generally agreed guidelines for the management of this disorder. Current therapeutic options to manage patients with factor X deficiency include fibrinolytic inhibitors, fresh-frozen plasma, and intermediate-purity factor IX concentrates (prothrombin complex concentrates). Recombinant VIIa (rVIIa) has been

used successfully to treat acquired factor X deficiency secondary to amyloidosis [32].

The need for replacement therapy is guided by the particular hemorrhagic episode. The biologic half-life of factor X is 20–40 h [33], so an adequate level can be achieved with repeated infusions. Factor levels of 10–20 IU/dL are generally sufficient for hemostasis, even in the immediate postoperative period [34], although some recent data suggest that levels of 5 IU/dL may be sufficient for adequate hemostasis [35].

Mucosal bleeding

Tranexamic acid

Tranexamic acid, a fibrinolytic inhibitor, may be of value in the management of patients with factor X deficiency. In practice, 10 mL of a 5% solution of tranexamic acid is used as a mouthwash every 8 h. However, this is not commercially available and therefore will need to be formulated by the hospital pharmacy. In women with menorrhagia, tranexamic acid 15 mg/kg 8 hourly (in practice 1 g 6–8 hourly) may be effective when taken for the duration of the menstrual period.

Fibrin glue

Fibrin glue can be effective in facilitating local hemostasis. Currently, two products are licensed for use in the UK—Tisseel® (Baxter) and Quixil® (Omrix)—although the latter is licensed for use only in liver surgery [36].

Fresh-frozen plasma

Fresh-frozen plasma (FFP) has been successfully used to manage patients with factor X deficiency. A dose of 20 mL/kg followed by 3–6 mL/kg twice daily is recommended, aiming to keep X:C trough levels above 10–20 IU/dL. This commonly requires large volumes of FFP and problems with fluid overload may be encountered. A virally inactivated FFP should be used.

Prothrombin complex concentrates

In many centers, prothrombin complex concentrates (PCCs) are used to manage patients with factor X deficiency, particularly severe factor X deficiency. The calculated required dosage for treatment is based on the empirical finding that 1 IU of factor X per kilogram of body weight raises the factor X level by 1.5% of normal. Tranexamic acid is generally avoided with PCCs because of the risk of thrombosis. The half-life of factor X is 20–40 h and daily treatment is not usually required. However, in cases where replacement therapy is given, levels should be monitored on a daily basis. In children, the biologic half-life of factor X may be shortened and fall-off studies may be required to establish an appropriate dosing regimen [35].

Management of an acute bleed in patients with severe factor X deficiency

No specific factor X concentrates are currently available and PCCs are probably the treatment of choice at present.

Fibrin glue

May be effective in facilitating local hemostasis.

Fresh-frozen plasma

In cases where PCCs are not available or are contraindicated, FFP can be used and is usually given as a loading dose of 10–20 mL/kg, followed by 3–6 mL/kg twice daily [34], aiming to keep X:C trough levels above the 10–20 IU/dL needed for effective hemostasis. A virally inactivated plasma should be used.

Prothrombin complex concentrates

PCCs are the treatment of choice for patients with severe factor deficiency. However, PCCs should be used with caution, if at all, in patients with concomitant liver disease, large hematomas, major trauma or antithrombin deficiency, or the neonate, because of the risk of precipitating a thrombosis [37].

Recombinant factor VIIa

Recombinant factor VIIa (rFVIIa) has been used to treat amyloid-associated factor X deficiency [32] but the data on its use in patients with inherited factor X deficiency is limited. Adequate levels of factor X appear to be important for the action of rFVIIa, and therefore in severe factor X deficiency rFVIIa may be ineffective [38].

Management of surgery in patients with severe factor X deficiency

Surgery in individuals with severe factor X deficiency (factor X < 1 U/dL) has been successfully performed following infusion of either FFP or PCCs. In the case of FFP, a level of 35 U/dL was achieved prior to surgery and factor X levels were maintained above 20 U/dL in the postoperative period with no bleeding reported [39]. A factor X level of 20 U/dL appears to be sufficient for efficient hemostasis. However, a recent report has shown that lower factor X levels, in the region of 0.05 IU/mL (5 IU/dL) achieves adequate hemostasis [35]. Similarly, data using the thrombogram suggest that factor X levels of 10 U/dL may be effective [25].

Factor X prophylaxis

Prophylaxis in patients with severe factor X deficiency using PCCs has been reported [40–43]. The level of factor X required

to achieve hemostasis, and therefore the dose/frequency of prophylaxis, is unclear. Pharmacokinetic studies can be invaluable in such cases to guide the dose/frequency of administration [43]. A dosage of 50–70 U/kg of a PCC every 3 days may be a sensible starting point.

In the majority of cases, the bleeding episodes are prevented without any serious treatment-related complication.

Management of severe factor X deficiency in pregnancy

Factor X levels increase during pregnancy [44], but women with severe factor X deficiency and a history of adverse outcome in pregnancy may benefit from aggressive replacement therapy [45]. However, the potential for thrombosis associated with replacement therapy must be considered.

Management of the neonate with severe factor X deficiency

In families in whom both parents are known to have factor X deficiency, pregnancy and delivery should be managed in such a way as to minimize the potential risk of bleeding both to the mother and baby. This requires close liaison with the obstetric unit including obstetric anesthetists, and a management plan should be prepared for the delivery and subsequent investigation of the neonate. A factor X assay should be performed prior to delivery. At birth, a cord blood sample should be taken for factor X assay.

Cranial ultrasound should be undertaken in severely affected neonates because of the increased risk of ICH. Prophylaxis during the neonatal period may be necessary in severely affected neonates.

Management of moderate factor X deficiency (FX:C > 2 U/dL)

Patients with factor X levels >10 U/dL or a lower level and no significant bleeding history (despite hemostatic challenges) require no replacement therapy. However, the nature of the surgery and any bleeding history in relation to previous hemostatic challenges must be considered.

Gene therapy for factor X deficiency

To date, there have been no trials of gene therapy in patients with severe factor X deficiency. Liver transplantation in one case of factor X deficiency has been reported [46] and was associated with normal factor X levels within 72 h of transplantation.

References

1 Hougie C, Barrow EM, Graham JB. The Stuart factor: a hitherto unrecognized blood coagulation factor. *Bibl Haematol* 1958; **7**: 336–40.

2 Telfer TP, Denson KW, Wright DR. A new coagulation defect. *Br J Haematol* 1956; **2**: 308–16.

3 Duckert F, Fluckiger P, Koller F. Le role du facteur X dans la formation de la thromboplastine sanguine. *Rev Hematol* 1954; **9**: 489–92.

4 Duckert F, Fluckiger P, Koller F. Le role de facteur X dans la formation de la thromboplastine sanguine. *Revue d'Hematologie* 1954; **9**: 489–92.

5 Scambler PJ, Williamson R. The structural gene for human coagulation factor X is located on chromosome 13q34. *Cytogenet Cell Genet* 1985; **39**: 231–3.

6 Davidson CJ, Hirt RP, Lal K, *et al.* Molecular evolution of the vertebrate blood coagulation network. *Thromb Haemost* 2003; **89**: 420–8.

7 Degen SJ, Davie EW. The prothrombin gene and serine proteinase evolution. *Ann NY Acad Sci* 1986; **485**: 66–72.

8 Patthy L. Evolution of the proteases of blood coagulation and fibrinolysis by assembly from modules. *Cell* 1985; **41**: 657–63.

9 Fung MR, Hay CW, MacGillivray RT. Characterization of an almost full-length cDNA coding for human blood coagulation factor X. *Proc Natl Acad Sci USA* 1985; **82**: 3591–5.

10 Brandstetter H, Kuhne A, Bode W, *et al.* X-ray structure of active site-inhibited clotting factor Xa. Implications for drug design and substrate recognition. *J Biol Chem* 1996; **271**: 29988–92.

11 Kamata K, Kawamoto H, Honma T, Iwama T, Kim SH. Structural basis for chemical inhibition of human blood coagulation factor Xa. *Proc Natl Acad Sci USA* 1998; **95**: 6630–5.

12 Maignan S, Guilloteau JP, Pouzieux S, *et al.* Crystal structures of human factor Xa complexed with potent inhibitors. *J Med Chem* 2000; **43**: 3226–32.

13 Peyvandi F, Menegatti M, Santagostino E, *et al.* Gene mutations and three-dimensional structural analysis in 13 families with severe factor X deficiency. *Br J Haematol* 2002; **117**: 685–92.

14 Philippou H, Adami A, Boisclair MD, Lane DA. An ELISA for factor X activation peptide: application to the investigation of thrombogenesis in cardiopulmonary bypass. *Br J Haematol* 1995; **90**: 432–7.

15 Schulman S, Tjonnfjord GE, Wallensten R, Martinowitz U, Kenet G. Continuous infusion of recombinant factor VIIa for surgery in patients with deficiency of factor VII. *Thromb Haemost* 2005; **94**: 1177–80.

16 Jesty J, Lorenz A, Rodriguez J, Wun TC. Initiation of the tissue factor pathway of coagulation in the presence of heparin: control by antithrombin III and tissue factor pathway inhibitor. *Blood* 1996; **87**: 2301–7.

17 Epstein DJ, Bergum PW, Bajaj SP, Rapaport SI. Radioimmunoassays for protein C and factor X. Plasma antigen levels in abnormal hemostatic states. *Am J Clin Pathol* 1984; **82**: 573–81.

18 Girolami A, Scapin M, Scarparo P, Vettore S. Different genotypes are responsible for the normal Russell viper venom assays seen in some cases of congenital factor X deficiency. *Am J Hematol* 2008; **83**: 884–5.

19 Girolami A, Molaro G, Lazzarin M, Scarpa R. [A new congenital hemorrhagic coagulation disorder probably due to the presence of an abnormal factor X. Preliminary study]. *Minerva Med* 1969; **60**: 4939–49.

20 Girolami A, Molaro G, Lazzarin M, Scarpa R, Brunetti A. A "new" congenital haemorrhagic condition due to the presence of an abnormal factor X (factor X Friuli): study of a large kindred. *Br J Haematol* 1970; **19**: 179–92.

21 Girolami A, Vicarioto M, Ruzza G, Cappellato G, Vergolani A. Factor X Padua: a "new" congenital factor X abnormality with a

defect only in the extrinsic system. *Acta Haematol* 1985; **73**: 31–6.
22 Girolami A, Scarparo P, Scandellari R, Allemand E. Congenital factor X deficiencies with a defect only or predominantly in the extrinsic or in the intrinsic system: a critical evaluation. *Am J Hematol* 2008; **83**: 668–71.
23 Williams MD, Chalmers EA, Gibson BE. The investigation and management of neonatal haemostasis and thrombosis. *Br J Haematol* 2002; **119**: 295–309.
24 Herrmann FH, Auerswald G, Ruiz-Saez A, *et al*. Factor X deficiency: clinical manifestation of 102 subjects from Europe and Latin America with mutations in the factor 10 gene. *Haemophilia* 2006; **12**: 479–89.
25 Al Dieri R, Peyvandi F, Santagostino E, *et al*. The thrombogram in rare inherited coagulation disorders: its relation to clinical bleeding. *Thromb Haemost* 2002; **88**: 576–82.
26 Fair DS, Edgington TS. Heterogeneity of hereditary and acquired factor X deficiencies by combined immunochemical and functional analyses. *Br J Haematol* 1985; **59**: 235–48.
27 Dewerchin M, Liang Z, Moons L, *et al*. Blood coagulation factor X deficiency causes partial embryonic lethality and fatal neonatal bleeding in mice. *Thromb Haemost* 2000; **83**: 185–90.
28 Tai SJ, Herzog RW, Margaritis P, *et al*. A viable mouse model of factor X deficiency provides evidence for maternal transfer of factor X. *J Thromb Haemost* 2008; **6**: 339–45.
29 Scambler PJ, Williamson R. The structural gene for human coagulation factor X is located on chromosome 13q34. *Cytogenet Cell Genet* 1985; **39**: 231–3.
30 Brown S, Gersen S, Anyane-Yeboa K, Warburton D. Preliminary definition of a "critical region" of chromosome 13 in q32: report of 14 cases with 13q deletions and review of the literature. *Am J Med Genet* 1993; **45**: 52–9.
31 Korsan-Bengsten LHP, Ygge J. Acquired factor X deficiency in a patient with amyloidosis. *Thromb Diath Haemorh* 1962; **7**: 558–66.
32 Boggio L, Green D. Recombinant human factor VIIa in the management of amyloid-associated factor X deficiency. *Br J Haematol* 2001; **112**: 1074–5.
33 Roberts HR, Lechler E, Webster WP, Penick GD. Survival of transfused factor X in patients with Stuart disease. *Thromb Diath Haemorrh* 1965; **13**: 305–13.
34 Knight RD, Barr CF, Alving BM. Replacement therapy for congenital factor X deficiency. *Transfusion* 1985; **25**: 78–80.
35 McMahon C, Smith J, Goonan C, Byrne M, Smith OP. The role of primary prophylactic factor replacement therapy in children with severe factor X deficiency. *Br J Haematol* 2002; **119**: 789–91.
36 UKHCDO. Guidelines on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders. *Haemophilia* 2003; **9**: 1–23.
37 Kohler M. Thrombogenicity of prothrombin complex concentrates. *Thromb Res* 1999; **95**(Suppl. 1): S13–17.
38 Allen GA, Monroe DM, 3rd, Roberts HR, Hoffman M. The effect of factor X level on thrombin generation and the procoagulant effect of activated factor VII in a cell-based model of coagulation. *Blood Coagul Fibrinolysis* 2000; **11**(Suppl. 1): S3–7.
39 Knight RD, Barr CF, Alving BM. Replacement therapy for congenital Factor X deficiency. *Transfusion* 1985; **25**: 78–80.
40 Auerswald G. Prophylaxis in rare coagulation disorders—factor X deficiency. *Thromb Res* 2006; **118**(Suppl. 1): S29–31.
41 Kouides PA, Kulzer L. Prophylactic treatment of severe factor X deficiency with prothrombin complex concentrate. *Haemophilia* 2001; **7**: 220–3.
42 Todd T, Perry DJ, Hayman E, Lawrence K, Gattens M, Baglin T. Severe factor X deficiency due to a homozygous mutation (Cys364Arg) that disrupts a disulphide bond in the catalytic domain. *Haemophilia* 2006; **12**: 621–4.
43 McMahon C, Smith J, Goonan C, Byrne M, Smith OP. The role of primary prophylactic factor replacement therapy in children with severe factor X deficiency. *Br J Haematol* 2002; **119**: 789–91.
44 Condie RG. A serial study of coagulation factors XII, XI and X in plasma in normal pregnancy and in pregnancy complicated by pre-eclampsia. *Br J Obstet Gynaecol* 1976; **83**: 636–9.
45 Kumar M, Mehta P. Congenital coagulopathies and pregnancy: report of four pregnancies in a factor X-deficient woman. *Am J Hematol* 1994; **46**: 241–4.
46 McCarthy M, Gane E, Pereira S, *et al*. Liver transplantation for haemophiliacs with hepatitis C cirrhosis. *Gut* 1996; **39**: 870–5.
47 Leytus SP, Foster DC, Kurachi K, Davie EW. Gene for human factor X: a blood coagulation factor whose gene organization is essentially identical with that of factor IX and protein C. *Biochemistry* 1986; **25**: 5098–102.

53 Factor XI deficiency

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History

Factor XI (FXI) deficiency was first described in 1953 by Rosenthal *et al*. [1] as a new type of hemophilia, later termed hemophilia C. Its presence in two sisters and their maternal uncle was interpreted as an indication that the mode of inheritance was autosomal dominant. However, a seminal study in 1961 clearly established that transmission of the disorder was autosomal [2], and distinguished between patients with major FXI deficiency, with an activity of less than 20 U/dL, and patients with minor deficiency, with an activity of 30–60 U/dL. This study, as well as a later study [3], also delineated that FXI deficiency was particularly common in Jews.

In the classic "waterfall" or "cascade" scheme of coagulation, designed in 1964, FXI was assigned a role in the initial "contact phase" of the intrinsic system. It was shown that negatively charged surfaces trigger activation of factor XII (FXII), later found to occur in the presence of prekallikrein (PK) and high-molecular-weight kininogen (HK), and that FXIIa, in turn, activates FXI. FXIa then activates factor IX (FIX) in the presence of Ca^{2+} ions, which leads through additional reactions to the generation of thrombin. Yet this sequence of reactions was hard to reconcile with the clinical observations which indicated that patients with severe deficiencies of FXII, PK, or HK had no bleeding tendency, whereas patients with FXI deficiency exhibited a significant bleeding tendency, particularly following trauma. In 1991 two groups of researchers showed that FXI was activated by thrombin, thereby bypassing the initial contact reactions [4,5]. These observations enabled design of a revised scheme of coagulation [4] in which FXII, PK, and HK play no role, while FXI is important for generating thrombin following its initial formation by the tissue factor–factor VII pathway (see Chapter 1). However, recent evidence disputes this and the role of the contact system is being re-evaluated [6].

Biochemical features and function of factor XI

Factor XI is a 160-kDa glycoprotein that consists of two identical polypeptide subunits of 80-kDa linked by a disulfide bond. Each subunit contains 607 amino acids organized in a heavy chain with four tandem repeats of 90 or 91 amino acids, designated "apple domains," and a light chain in which a serine protease domain is located. The first apple domain contains the binding sites for HK, with which FXI circulates as a complex, and for prothrombin. Apple 3 contains the binding sites for platelets and FIX, and apple 4 harbors the binding site for FXII and is important for the dimerization of the subunits [7,8].

The gene encoding for FXI (GenBank M18295) consists of 15 exons and 14 introns and is located on chromosome 4q34–35 close to the gene for PK [9]. FXI is synthesized in the liver. The physiologic activator of FXI is thrombin, which converts zymogen FXI to a serine protease (FXIa) by cleavage of the Arg369–Ile370 bond, giving rise to a 47-kDa heavy chain and a 33-kDa light chain. Evidence suggests that this reaction is greatly accelerated when FXI is bound to activated platelets in the presence of prothrombin and Ca^{2+} ions or HK and Zn^{2+} ions [10]. Conceivably, one of the FXI subunits binds to the platelet membrane while the other subunit binds to FIX [7], thereby enabling efficient activation of FXI by thrombin and then activation of FIX by FXIa. FXIa initially cleaves FIX at the Arg146–Ala147 bond in the presence of Ca^{2+} ions and subsequently an Arg180–Val181 bond yielding fully activated factor IXa and an activation peptide. FIXa then activates FX in the presence of factor VIIIa, negatively charged phospholipids and Ca^{2+} ions, and FXa in turn converts prothrombin to thrombin in the presence of factor Va, negatively charged phospholipids, and Ca^{2+} ions.

Factor XI activated by thrombin is essential for sustained thrombin generation, which is particularly important after clot formation. It also diminishes fibrinolysis as thrombin activates procarboxypeptidase B, also termed thrombin-activatable fibrinolysis inhibitor (TAFI). Activated TAFI removes terminal lysine residues from fibrin, the binding sites of plasminogen and tissue plasminogen activator thereby inhibiting fibrinolysis [11]. Factor XI can thus be regarded as a procoagulant and an indirect inhibitor of fibrinolysis. This conclusion is supported by the clinical observation that patients with severe FXI deficiency are specifically prone to bleeding when trauma is inflicted at sites where there is enhanced fibrinolytic activity [12]. FXIa is inhibited by antithrombin in the presence of heparin, protease nexin II, C_1-inhibitor, and protein C inhibitor.

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Inheritance and functional defect

Factor XI deficiency is inherited as an autosomal disorder. Homozygotes or compound heterozygotes have an FXI activity of <15 U/dL and heterozygotes have an activity range of 25–70 U/dL or are within normal limits. Vertical transmission of severe FXI deficiency or apparent dominance has been observed in Ashkenazi Jewish families but stems from matings between homozygotes and heterozygotes in this population, in which the prevalence of mutant genes and affected individuals is high [3]. In the vast majority of patients with FXI deficiency, FXI activity is concordant with antigenicity [13]. Only a few patients have so far been described with dysfunctional FXI (i.e., 100 U/dL antigenicity and <1 U/dL activity) [14].

Mutations

Three mutations in the FXI gene, termed types I, II, and III, were first described in 1989 in six Ashkenazi Jews who had severe FXI deficiency [15]. Type I mutation is a G to A change at the splice junction of the last intron of the gene, type II mutation is a G to T change in exon 5 leading to Glu117stop, and type III mutation is a T to C change in exon 9, giving rise to Phe283Leu substitution. Homozygotes for type II mutation have a mean FXI activity of 1.2 U/dL, homozygotes for type III mutation have a mean FXI activity of 9.7 U/dL, and compound heterozygotes for types II and III mutations have a mean activity of 3.3 U/dL [12]. Type II and III mutations are the predominant mutations causing FXI deficiency in Ashkenazi Jews [12,16]. Table 53.1 demonstrates that in 295 Jewish patients of various ethnic origins with severe FXI deficiency, 52% of the alleles harbored type II mutation, 46% type III mutation, 1% type I mutation, and 1% other mutations. Databases listing published mutations are available (http://www.isth.org, look under Registries/Databases/Mutations Causing Rare Bleeding Disorders, www.hgmd.org, and www.factorxi.com). Most mutations responsible for FXI deficiency are missense (111/165) associated with equal reduction in both activity and antigen. Ten mutations are reported with discrepant activity and antigen (www.factorxi.com). Expression of several missense mutations revealed impaired secretion of FXI from transfected cells [17,18]. For one of these mutations, the type III mutation (Phe283Leu) in apple 4, the impaired secretion was related to defective dimerization [17]. Several polymorphisms have also been described and used for analysis of haplotypes in populations in which FXI deficiency is prevalent [19,20].

Table 53.1 Molecular analysis in 295 unrelated Jewish patients with severe factor XI deficiency.

Mutant allele	*n*	%
Type II: Glu117Ter	306	51.86
Type III: Phe283Leu	271	45.93
Type I: IVS 14 + 1 (Ggt → Gat)	7	1.19
Type IV: 14-bp deletion (IVS 14/exon 15)	2	0.34
Gly555Glu	2	0.34
Tyr427Cys	1	0.17
Glu323Lys	1	0.17
Total	*590*	*100*

Prevalence and ethnic distribution

The highest prevalence of FXI deficiency has been observed in Ashkenazi Jews [3]. Among 531 individuals of Ashkenazi Jewish origin, the allele frequency of type II and type III mutations was 0.0217 and 0.0254, respectively [21]. Thus, 9.1% of subjects belonging to this ethnic group are predicted to be carriers of either mutation and 1 in 450 individuals (0.22%) is expected to have severe FXI deficiency. The Iraqi Jews, who represent the ancient gene pool of Jews from Babylonian times 2500 years ago, have only the type II mutation. Among 507 subjects, an allele frequency of 0.0167 was found, predicting heterozygosity in 1/30 individuals and homozygosity in 1/3600 individuals in the general Iraqi Jewish community [21]. Interestingly, among 382 Arabs, type II mutation was detected with an allele frequency of 0.0065. A study of intragenic polymorphisms in patients with severe FXI deficiency from all these ethnic groups enabled haplotype analysis, which disclosed distinct founder effects for the type II and type III mutations [20] (Figure 53.1). Based on the distribution of allelic variants at a microsatellite marker flanking the FXI gene (*D4S171*), the type II mutation was estimated to have occurred more than 120 generations ago, while the type III mutation was of a more recent origin [22] (Figure 53.2). Another cluster of patients with FXI deficiency was observed in Basques residing in southwestern France, and a recent study revealed that the predominant mutation in this population is Cys38Arg, with an allele frequency of 0.005 [19]. Another cluster of FXI-deficient patients harboring a C128X mutation was described in Caucasians living or originating in the UK [23]. Haplotype analysis for this mutation is consistent with a founder effect.

Factor XI deficiency has also been reported sporadically in other patients of English, African-American, German, Indian, Italian, Korean, Japanese, Chinese, Portuguese, Swedish, Yugoslav, Arab, and Iranian origin.

Bleeding manifestations in patients with severe deficiency

Spontaneous bleeding manifestations are rare in patients with severe FXI deficiency. The common presentation is an injury-related bleeding tendency, particularly at sites where tissues contain activators of the fibrinolytic system, such as the oral cavity, nose, tonsils, and urinary tract [12,24]. At other sites

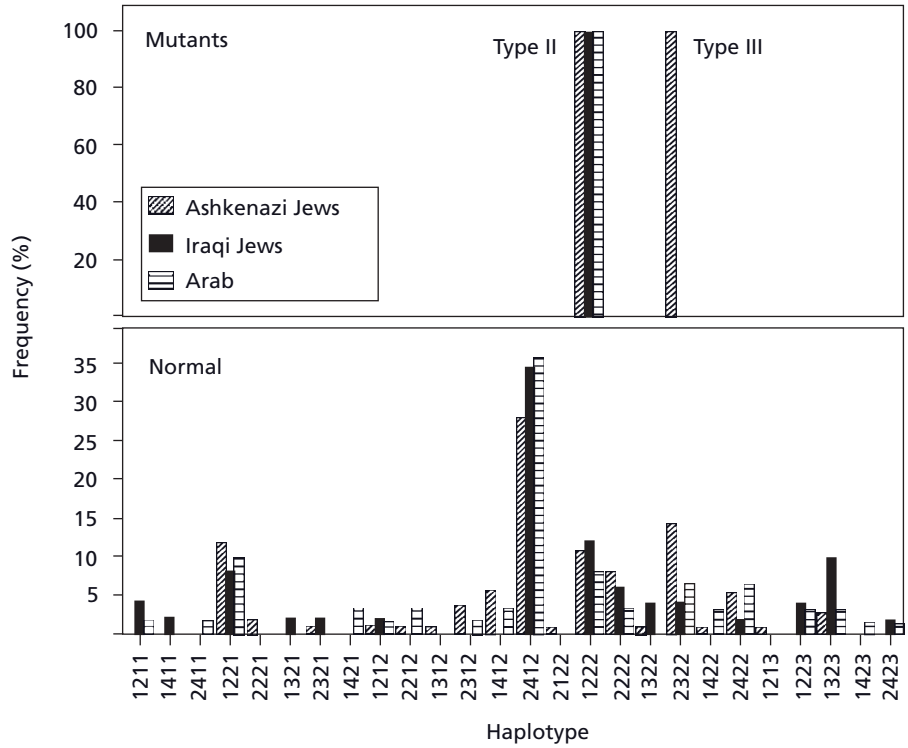

Figure 53.1 Frequency distribution of factor XI gene haplotypes observed in Ashkenazi Jews, Iraqi Jews, and Arabs. The numbers on the abscissa denote, from bottom to top, the allele numbers of polymorphisms in introns A, B, E, and M. For example, the haplotype designated 1–2–1–1 comprises allele 1 of intron A polymorphism, allele 2 of intron B polymorphism, allele 1 of intron E polymorphism, and allele 1 of intron M polymorphism. The lower and upper panels represent normal chromosomes and chromosomes bearing type II or type III mutations, respectively. Note that all chromosomes carrying the type II mutation are characterized by the same 1–2–2–2 haplotype that is observed in 8–12% of normal chromosomes. The chromosomes bearing the type III mutation are confined to Ashkenazi Jews, all characterized by haplotype 2–3–2–2. From Peretz *et al*. [20] with permission from the American Society of Hematology.

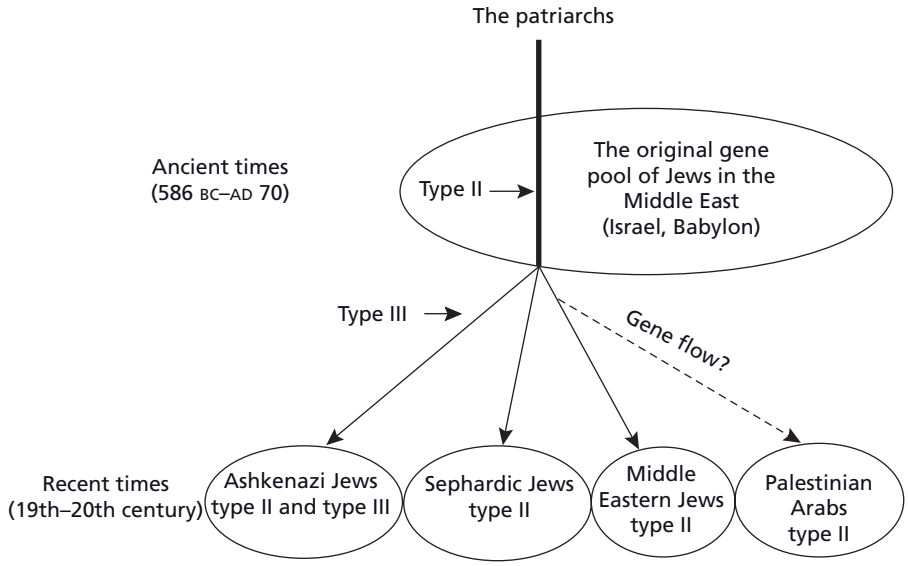

Figure 53.2 A simplified scheme showing the common origin of the three major segments of contemporary Jews and explaining the current distribution of the type II and type III mutations. The predicted time when type II and type III mutations occurred in the factor XI gene are indicated by horizontal arrows. We speculate that gene flow has been responsible for the transfer of type II mutation from Middle Eastern Jews to Palestinian Arabs after the settlement of Arabs in Israel in the 7th century AD. From Peretz *et al*. [20] with permission from the American Society of Hematology.

of trauma, such as orthopedic surgical wounds, appendicectomy, circumcision, or cuts in the skin, bleeding is less common. Postpartum hemorrhage occurs in only 20–30% of affected women [25,26]. Some patients with a very low level of FXI may not bleed at all following trauma [2], while in others the bleeding tendency varies in the same patient over time even when provoked by similar hemostatic challenges [12,25]. Bleeding can be brisk at the time of injury and persists unless treated, or can begin several hours following trauma.

Bleeding manifestations in heterozygotes

Some heterozygotes exhibit abnormal bleeding. In one study, bleeding was observed in 9/94 (9.6%) of patients who underwent surgical procedures, including tooth extractions, tonsillectomy, and nasal operation [2]. In another study, no bleeding was observed following urologic surgery unless FXI activity was 25 U/dL or less [27]. In contrast, two studies from the UK

and one from Iran [25,28,29] described injury-related bleeding in 48–60% of cases, as well as spontaneous bleeding manifestations. An assessment of the risk of bleeding in a large cohort of patients with severe and partial FXI deficiency yielded an odds ratio of 13 [confidence interval (CI) 3.8–45] for patients with severe FXI deficiency and an odds ratio of 2.6 (CI 0.8–9.0) for patients with partial deficiency [30]. In spite of these uncertainties, it can be concluded that patients with partial FXI deficiency may exhibit a bleeding tendency but the risk is substantially lower than in patients with severe FXI deficiency. Whatever the cause of the discrepancy among the studies, patients with partial FXI deficiency who have a bleeding history should be carefully examined for additional inherited disorders of hemostasis such as platelet dysfunction or von Willebrand disease (VWD), and for acquired hemostatic defects.

Thrombosis

The effect of FXI in promoting coagulation and inhibiting fibrinolysis could hypothetically predict that in patients with severe FXI deficiency, thrombosis would occur infrequently. However, unlike patients with severe hemophilia A or hemophilia B, in whom the incidence of acute myocardial infarction is significantly reduced, patients with severe FXI deficiency are not protected against such events. In a recent study of 96 adult patients with severe FXI deficiency, 16 (17%) have had an acute myocardial infarction, which occurred at median ages of 64.5 and 58 years in women and men, respectively [31]. The observed incidence of acute myocardial infarction in this cohort was not statistically different from the expected incidence in the general population. As anticipated, one or more of the conventional atherosclerotic risk factors was detected in 13 of the 16 patients who had an acute myocardial infarction (81%), whereas, among patients with severe FXI deficiency who had not experienced an acute myocardial infarction, the presence of these risk factors was significantly lower. In contrast, a reduced incidence of ischemic stroke has been observed in severe factor XI deficiency. A cohort of 115 patients over 45 years of age with severe FXI deficiency (level < 15 U/dL) was compared with an Israeli health survey of 9509 people. After correction for the common risk factors, the expected incidence of stroke was 8.56 but only one was observed [32].

Venous thromboembolism was not observed but anecdotal cases have been reported [33]. Conceivably, some protection against venous thromboembolism may be conferred by severe FXI deficiency because high levels of FXI constitute a risk factor for such venous thrombosis [34]. However, only a study of a very large number of patients with FXI deficiency would enable assessment of whether or not patients with severe FXI deficiency are protected against venous thromboembolism.

Association of factor XI deficiency with other disorders

Factor XI deficiency has been described in association with various other inherited bleeding disorders, e.g., VWD, platelet dysfunctions, and deficiencies of factors VII, VIII, and IX (for review see ref. 35). These associations are probably coincidental, although the probability of the common occurrence of combined deficiency of factors VII, IX, and XI, observed in five families, seems too remote to be coincidental. No mechanism for these exceptional families has been provided. Noonan syndrome and Gaucher disease have also been observed in patients with FXI deficiency. While the association between FXI deficiency and Noonan syndrome has not been explained, the association between Gaucher disease and FXI deficiency was shown to stem from the relatively high prevalence of the mutant genes of both disorders in Ashkenazi Jews, i.e., 1/19 for Gaucher disease and 1/11 for FXI deficiency. It was also demonstrated that the segregation of Gaucher disease and FXI deficiency in the same family was independent [36].

Development of inhibitors

Inhibitors to FXI have been described in patients with severe FXI deficiency. Fortunately, bleeding manifestations in such patients are not aggravated following inhibitor formation, but trauma or surgery presents a serious hemostatic challenge (see "Therapy"). In a recent study of 118 unrelated patients with severe FXI deficiency, seven were found to harbor an inhibitor [37]. All seven patients had received plasma replacement therapy prior to the development of the inhibitor and all were homozygous for the type II null allele, which is associated with extremely low levels of FXI. Of 84 patients with other genotypes, i.e., type III homozygotes or type II and type III compound heterozygotes (of whom 43 had received plasma), none was found to have an inhibitor to FXI. Patients with these genotypes have measurable levels of FXI in the range 2–15 U/dL. These observations suggest that only homozygotes or compound heterozygotes for two null alleles are prone to the development of an inhibitor following exposure to exogenous FXI. This study also revealed that the seven patients who developed an inhibitor were among 21 homozygotes for the type II mutation who had received plasma, which suggests that 33% of patients with almost no FXI have a predilection for development of an inhibitor. The antibodies isolated from patients with an inhibitor display various effects, i.e., impaired FXI activation by thrombin or by FXIIa, inhibition of binding of FXI to HK, and diminished activation of FIX by FXIa [37].

Diagnosis

Excessive bleeding following injury, such as tooth extraction, tonsillectomy, nose surgery, and urologic procedures, or an

incidental finding of a prolonged activated partial thromboplastin time (aPTT) are the common modes of presentation of FXI deficiency. Excessive menstrual bleeding is a recognized presentation of FXI deficiency. All patients with severe FXI deficiency (activity of less than 15 U/dL) exhibit an aPTT value that is more than two standard deviations above the normal mean [38]. Heterozygotes may have a slightly prolonged aPTT or values within the normal range but this is dependent upon the aPTT reagent. Similarly, FXI levels overlap with normal levels [3,25,29]. Therefore, for diagnosis of partial FXI deficiency in suspected heterozygotes, only genotyping for the responsible mutation can be diagnostic.

Because severe FXI deficiency can remain asymptomatic until injury is inflicted, it is desirable for all Ashkenazi Jews in need of surgery (particularly at sites of enhanced fibrinolysis) to undergo screening tests including aPTT. If a prolonged aPTT is obtained, FXI activity can be measured by an aPTT-based assay.

Therapy

Spontaneous bleeding is rare in patients with severe FXI deficiency, and when it occurs it is usually mild and terminates without therapy. Sometimes, spontaneous bleeding follows the inadvertent use of antiplatelet agents and discontinuation of their use solves the problem. Deliveries are infrequently complicated by excessive bleeding in patients with severe FXI deficiency and thus on-demand, rather than preventive, blood product therapy can be advocated [26]. Oral antifibrinolytic drugs are useful both for menorrhagia and for bleeding after childbirth when it occurs.

Surgery or trauma can be associated with excessive and prolonged bleeding unless treated properly. Consequently, careful evaluation of patients with severe FXI deficiency prior to surgery is indispensable, as well as meticulous planning of the procedure and the postsurgical course. The following are some considerations and guidelines:

1 The surgical procedure should be absolutely indicated.

2 Previous bleeding episodes and their severity should be taken into account.

3 A test for an inhibitor to FXI should be performed and its presence ruled out.

4 The prothrombin time and platelet count should be normal.

5 Use of antiplatelet drugs should be discontinued 1 week before surgery.

6 Both site and type of surgery are significantly related to the risk of bleeding and, hence, planning of surgery should be tailored accordingly. Examples include (i) patients in need of tooth extraction can be treated by tranexamic acid alone (see below); (ii) for prostatectomy and other lower urinary tract surgery, both blood component therapy and local flushing by saline containing tranexamic acid can be used; (iii) for nasal surgery and tonsillectomy, replacement therapy and parenteral tranexamic acid administration are advisable; (iv) for major surgery, plasma or FXI concentrate infusions should be targeted at trough FXI levels of 45 U/dL for approximately 10 days; for minor surgery a trough level of 30 U/dL during approximately 5 days is usually sufficient.

7 Assessment of the cardiovascular status of the patient is essential for two reasons: (i) when use of fresh-frozen plasma (FFP) is planned, the patient's incapacity to tolerate volume overload can be a serious impediment; (ii) compromised cardiovascular function confers a risk of thrombosis when an FXI concentrate is used.

8 Replacement therapy should be started prior to surgery and carefully monitored thereafter by assays of FXI activity.

9 Use of fibrin glue during surgery can significantly contribute to successful hemostasis.

10 Replacement therapy is usually by FFP. The main disadvantages of this mode of therapy are potential transmission of infectious agents, allergic reactions, and—as mentioned—volume overload. Treatment of FFP by solvent/detergent or by pasteurization has increased its safety, with preservation of FXI activity [39].

Two concentrates of FXI have been produced, one in the UK and the other in France, and both were found to be safe with regard to transmission of infectious agents. However, approximately 10% of patients who were treated by these products developed arterial thrombosis or venous thromboembolism which was fatal in several cases. As almost all patients who developed these unfortunate complications were elderly and had pre-existing cardiovascular disease [39], these products should be avoided in such patients or used with extreme caution. Caution should also be exercised in patients with prothrombotic states such as pregnancy and malignant disorders. Notwithstanding these limitations, FXI concentrates have been successfully used in many patients. Studies have shown a 90% recovery of FXI after infusion of these concentrates and a half-life of 46–52 h. The relatively small volume that needs to be infused, the excellent *in vivo* recovery of FXI, and the extended half-life of FXI substantially facilitate therapy.

For several types of minor surgery, such as tooth extractions and skin biopsy, there is no need for replacement therapy. In 19 patients with severe FXI deficiency who have had a history of bleeding following tooth extractions or trauma, tooth extractions were uneventfully performed under treatment with tranexamic acid alone started 12 h prior to surgery and continued until 7 days after surgery [40]. Fibrin glue can also be used in such cases and in patients undergoing resection of skin lesions.

The approach to patients with partial FXI deficiency who need surgery varies among centers. Excessive bleeding following surgery in some patients with FXI levels of approximately 50 U/dL is used to support the view that such patients need replacement therapy during surgery [29]. Other observations of patients who underwent uneventful prostatectomy with a level of 30 U/dL support the view that replacement therapy is unnecessary during most surgical procedures in patients with

partial FXI deficiency [27]. Notwithstanding these inconsistencies, a reasonable practice in patients with partial FXI deficiency can be:

1 Obtain a detailed history of bleeding.

2 If a clear history of bleeding tendency is obtained, perform a thorough investigation of other potential inherited or acquired hemostatic disorders; abnormal results should be taken into account in the planning of surgery.

3 Use tranexamic acid and/or fibrin glue when there is a bleeding history or when high-risk surgery such as prostatectomy is planned.

4 Use replacement therapy in patients with an unequivocal bleeding tendency (after ruling out other hemostatic defects) aiming at a trough FXI level of 45 U/dL for 5 days after surgery.

Surgery in patients who have developed an inhibitor to FXI presents a great challenge. When the titer of the inhibitor is very low, use of an FXI concentrate can suffice, but an anamnestic reaction is to be expected. A one-time low dose of recombinant factor VIIa (rFVIIa) given during surgery and prolonged therapy by tranexamic acid have been successfully used in two such patients who underwent major surgery [41].

References

1 Rosenthal R, Dreskin O, Rosenthal N. New hemophilia-like disease caused by deficiency of a third plasma thromboplastin factor. *Proc Soc Exp Biol Med* 1953; **82**: 171–4.

2 Rapaport SI, Proctor RR, Patch NJ, Yettra M. The role of inheritance of PTA deficiency: evidence for the existence of major PTA deficiency and minor PTA deficiency. *Blood* 1961; **18**: 149–65.

3 Seligsohn U. High gene frequency of factor XI (PTA) deficiency in Ashkenazi Jews. *Blood* 1978; **516**: 1223–8.

4 Gailani D, Broze GJ. Factor XI activation in a revised model of blood coagulation. *Science* 1991; **253**: 909–12.

5 Naito K, Fujikawa K. Activation of human blood coagulation factor XI independent of factor XII. Factor XI is activated by thrombin and factor XIa in the presence of negatively charged surfaces. *J Biol Chem* 1991; **266**: 7353–8.

6 Blat Y, Seiffert D. A renaissance for the contact system in blood coagulation? *Thromb Hemost* 2008; **99**: 457–60.

7 Gailani D, Ho D, Sun MF, *et al.* Model for a factor IX activation complex on blood platelets: dimeric conformation of factor XIa is essential. *Blood* 2001; **97**: 3117–22.

8 Papagrigoriou E, McEwan PA, Walsh PN, Emsley J. Crystal structure of the efactor XI zymogen reveals a pathway for transactivation. *Nature Struct Mol Biol* 2006; **13**: 557–8.

9 Kato A, Asakai R, Davie EW, Aoki N. Factor XI gene (*F11*) is located on the distal end of the long arm of human chromosome 4. *Cytogenet Cell Genet* 1989; **52**: 77–8.

10 Baglia FA, Badellino KO, Li CQ, *et al.* Factor XI binding to the platelet glycoprotein Ib-IX-V complex promotes factor XI activation by thrombin. *J Biol Chem* 2002; **277**: 1662–8.

11 Bouma BN, Marx PF, Mosnier LO, Meijers JC. Thrombin-activatable fibrinolysis inhibitor (TAFI, plasma procarboxypeptidase B, procarboxypeptidase R, procarboxypeptidase U). *Thromb Res* 2001; **101**: 329–54.

12 Asakai R, Chung DW, Davie EW, Seligsohn U. Factor XI deficiency in Ashkenazi Jews in Israel. *N Engl J Med* 1991; **3253**: 153–8.

13 Saito H, Ratnoff OD, Bouma BN, Seligshon U. Failure to detect variant (CRM+) plasma thromboplastin antecedent (factor XI) molecules in hereditary plasma thromboplastin antecedent deficiency: a study of 125 patients in several ethnic backgrounds. *J Lab Clin Med* 1985; **106**: 718–22.

14 Zivelin A, Ogawa T, Bulvik S, *et al.* Severe factor XI deficiency caused by a Gly555 to Glu mutation (factor XI—Glu555): a cross-reactive material positive variant defective in factor XI activation. *J Thromb Haemost* 2004; **2**: 1782–9.

15 Asakai R, Chung DW, Ratnoff OD, Davie EW. Factor XI (plasma thromboplastin antecedent) deficiency in Ashkenazi Jews is a bleeding disorder that can result from three types of point mutations. *Proc Natl Acad Sci USA* 1989; **86**: 7667–71.

16 Hancock JF, Wieland K, Pugh RE, *et al.* A molecular genetic study of factor XI deficiency. *Blood* 1991; **77**: 1942–8.

17 Meijers JC, Mulvihill ER, Davie EW, Chung DW. Apple four in human blood coagulation factor XI mediates dimer formation. *Biochemistry* 1992; **31**: 4680–4.

18 Pugh RE, McVey JH, Truddenham EG, Hancock JF. Six point mutations that cause factor XI deficiency. *Blood* 1995; **85**: 1509–16.

19 Zivelin A, Bauduer F, Ducout L, *et al.* Factor XI deficiency in French Basques is caused predominantly by an ancestral Cys38Arg mutation in the factor XI gene. *Blood* 2002; **99**: 2448–54.

20 Peretz H, Mulai A, Usher S, *et al.* The two common mutations causing factor XI deficiency in Jews stem from distinct founders: one of ancient Middle Eastern origin and another of more recent European origin. *Blood* 1997; **90**: 2654–9.

21 Shpilberg O, Peretz H, Zivelin A, *et al.* One of the two common mutations causing factor XI deficiency in Ashkenazi Jews (type II) is also prevalent in Iraqi Jews, who represent the ancient gene pool of Jews. *Blood* 1995; **85**: 429–32.

22 Goldstein DB, Reich DE, Bradman N, *et al.* Age estimates of two common mutations causing factor XI deficiency: recent genetic drift is not necessary for elevated disease incidence among Ashkenazi Jews. *Am J Hum Genet* 1999; **64**: 1071–5.

23 Bolton-Maggs PHB, Peretz H, Butler R, *et al.* A common ancestral mutation (C128X) occurring in 11 non-Jewish families from the UK with factor XI deficiency. *J Thromb Haemost* 2004; **2**: 918–24.

24 Salomon O, Steinberg DM, Seligsohn U. Variable bleeding manifestations characterize different types of surgery in patients with severe factor XI deficiency enabling parsimonious use of replacement therapy. *Haemophilia* 2006; **12**: 490–3.

25 Bolton-Maggs PH, Patterson DA, Wensley RT, Tuddenham EG. Definition of the bleeding tendency in factor XI-deficient kindreds —a clinical and laboratory study. *Thromb Haemost* 1995; **73**: 194–202.

26 Salomon O, Steinberg DM, Tamarin I, *et al.* Plasma replacement therapy during labor is not mandatory for women with sever factor XI deficiency. *Blood Coagul Fibrinolysis* 2005; **16**: 37–41.

27 Sidi A, Seligsohn U, Jonas P, Many M. Factor XI deficiency: detection and management during urological surgery. *J Urol* 1978; **119**: 528–30.

28 Peyvandi F, Lak M, Mannucci PM. Factor XI deficiency in Iranians: its clinical manifestations in comparison with those of classic hemophilia. *Haematologica* 2002; **87**: 512–14.

29 Bolton-Maggs PH, Young Wan-Yin B, McCraw AH, *et al.* Inheritance and bleeding in factor XI deficiency. *Br J Haematol* 1988; **69**: 521–8.

30 Brenner B, Laor A, Lupo H, *et al.* Bleeding predictors in factor-XI-deficient patients. *Blood Coagul Fibrinol* 1997; **8**: 511–15.
31 Salomon O, Steinberg DM, Dardik R, *et al.* Inherited factor XI deficiency confers no protection against acute myocardial infarction. *J Thromb Haemost* 2003; **1**: 658–61.
32 Salomon O, Steinberg DM, Koren-Morag N, *et al.* Reduced incidence of ischemic stroke in patients with severe factor XI deficiency. *Blood* 2008; **111**: 4113–17.
33 Brodsky JB, Burgess GE. Pulmonary embolism with factor XI deficiency. *JAMA* 1975; **234**: 1156–7.
34 Meijers JC, Tekelenburg WL, Bouma BN, *et al.* High levels of coagulation factor XI as a risk factor for venous thrombosis. *N Engl J Med* 2000; **342**: 696–701.
35 Gailani D, Broze GJ. Factor XI and the contact system. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds.) *The Metabolic and Molecular Basis of Inherited Diseases*, 8th edn. New York: McGraw-Hill Publishing, 2000: 4433–4453.
36 Seligsohn U, Zitman D, Many A, Klibansky C. Coexistence of factor XI (plasma thromboplastin antecedent) deficiency and Gaucher's disease. *Isr J Med Sci* 1976; **12**: 1448–52.
37 Salomon O, Ziveiln A, Livnat T, *et al.* Prevalence, causes, and characterization of factor XI inhibitors in patients with inherited factor XI deficiency. *Blood* 2003; **101**: 4783–88.
38 Seligsohn U, Modan M. Definition of the population at risk of bleeding due to factor XI deficiency in Ashkenazic Jews and the value of activated partial thromboplastin time in its detection. *Isr J Med Sci* 1981; **17**: 413–15.
39 Bolton-Maggs PHB. Factor XI deficiency and its management. *Haemophilia* 2000; **6**: 100–9.
40 Berliner S, Horowitz I, Martinowitz U, *et al.* Dental surgery in patients with severe factor XI deficiency without plasma replacement. *Blood Coagul Fibrinol* 1992; **3**: 465–8.
41 Livnat T, Tamarin I, Mor Y, *et al.* Recombinant activated factor VII and tranexamic acid are hemostatically effective during major surgery in factor XI-deficient patients with inhibitor antibodies. *Thromb Haemost* 2009; **102**: 487–92.

54 Factor XIII deficiency

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Introduction

Factor XIII (FXIII) is a transglutaminase enzyme that was first discovered as a clotting protein in the coagulation cascade. However, recent literature describes multiple roles for this factor, including the ability to cross-link proteins in the plasma, vascular matrix, endothelial cells, platelets, and monocytes. In addition to maintaining normal hemostasis, FXIII plays a role in atherosclerosis, wound healing, inflammation, and pregnancy [1–3].

FXIII circulates in plasma as a tetramer protein (FXIII-A_2B_2) held together by noncovalent bonds. FXIII has a molecular weight of 325 kDa with two catalytic A subunits (FXIII-A_2) of 83 kDa and two noncatalytic B subunits or carrier subunits (FXIII-B_2) of 79 kDa [4–6]. Refer to Table 54.1 for the International Society of Thrombosis Hemostasis (ISTH) recommended nomenclature for the various forms of FXIII used for research publications [7].

Intracellular FXIII-A_2 exists as a homodimer and can be found in platelets, monocytes, tissue macrophages, and placenta. Nearly half of the total amount of FXIII in whole blood can be found in association with circulating platelets [8]. Intracellular FXIII-A_2 is released during hemostasis and binds to FXIII-B_2 to form the tetramer complex (FXIII-A_2B_2), which functions to stabilize the fibrin clot. Platelet FXIII can also enhance clot formation by cross-linking platelets to clotting proteins such as von Willebrand factor, factor V, and fibrinogen [9,10]. FXIII-A contains the activation site of the enzyme and is synthesized by hematopoietic cells such as monocytes and megakaryocytes with some production from hepatocytes as well [11–13]. Intracellular platelet FXIIIA has also been found to play a role in lamellapodia formation and spreading on a fibrinogen-coated surface [14].

Approximately 50% of total FXIII-B can be found in plasma as a free form with the remainder existing in the heterotetrameric form (FXIII-A_2B_2) bound to FXIII-A_2 [15]. The main role of FXIII-B is to protect FXIII-A_2 from proteolytic degradation and inactivation, thereby extending the time FXIII-A_2 remains in circulation. Another critical role of FXIII-B is the localization of FXIII to the polymerizing fibrin chains to facilitate cross-linking. FXIII-B is primarily produced in the liver [12,16]. Plasma FXIII-B specifically binds to the γ′ chain of fibrinogen type 2 [17]. Fibrinogen 2 acts as a carrier for FXIII in plasma and helps to downregulate potential cross-linking activity [18].

Structure/function

FXIII plays an important role in hemostasis by catalysing the cross-linking of fibrin as well as enhancing fibrin interaction with a variety of integrins within the platelet membrane and matrix proteins throughout thrombus formation. In aggregate, these biologic processes strengthen and stabilize the blood clot.

X-ray crystallography showed that FXIII-A subunit contains an activation peptide, a β-sandwich, a catalytic core, and barrel 1 and barrel 2 domains [19,20]. The activation peptide, composed of 37 amino acids, restricts the access of the substrate to the active site cysteine [21]. Activated FXIII (FXIIIa) binds to fibrin through the α-C-domain enhancing dissociation of the FXIII subunits [21].

The B-subunit is composed of 10 repeated Sushi or glycoprotein 1 domains [22]. Each Sushi domain has two disulfide bridges that sustain its tertiary structure. As stated before, the B subunit binds to fibrinogen 2 γ chain and helps regulate cross-linking activity [17].

Souri *et al.* studied the function of FXIII-B by expressing recombinant FXIII-B in baculoviruses [23]. The results revealed specific roles for the individual Sushi components: the first Sushi domain was involved in the binding of FXIII-B to FXIII-A whereas the fourth and ninth Sushi domains were responsible for the FXIII-B homodimer assembly. The study also confirmed that rFXIII-B in the heterotetramer form (FXIII-A_2B_2) protected FXIII-A from proteolytic digestion [23].

Activation of FXIII begins with thrombin cleavage of A-subunits and requires a critical mass of fibrin polymers. Thrombin mediates cleavage of the activation peptides (AP-FXIII) of FXIII-A from the N-terminus at position 37 [24–26]. In the presence of Ca^{2+} and fibrin, the B subunits dissociate from the A subunits causing a conformational change exposing the active site [27] (Figure 54.1). This active site contains a cysteine residue (Cys311) which is found within the sequence Tyr–Gly–Gln–Cys–Trp. Activated FXIII-A_2 (FXIII-A_2*) will then cross-link fibrin through ε-amino(γ-glutamyl) lysine

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isopeptide bond [28,29]. The B subunits dissociate while the majority of A subunits remain bound to fibrin [30].

Thrombin catalyzes formation of γ-glutamyl-ε lysl bonds between fibrin monomers, resulting in a fibrin meshwork that is insoluble in mild acids and urea.

Activated FXIII cross-links fibrin polymers present at very low concentrations, before a clot is visible [21]. FXIIIa can also crosslink fibrinogen but at a much slower rate [31,32].

The activation rate of intracellular factor XIII by thrombin is more rapid than plasma FXIII thrombin conversion to XIIIa. Studies have shown a lag time between the first steps of plasma FXIII activation: thrombin cleavage and exposure of the active site [33,34]. This delay corresponds to the amount of time needed for the B subunit to dissociate from activated plasma FXIII. In contrast to plasma FXIII activation, platelet FXIII activation depends on high levels of intracellular Ca^{2+} triggering the protein to undergo a nonproteolytic conformation change to its active form.

In addition to binding fibrin(ogen), FXIII cross-links numerous substrates involved in hemostasis and antifibrinolysis. FXIII incorporates antifibrinolytic proteins such as α_2-antiplasmin, plasminogen activator inhibitor 2 (PAI-2), and thrombin activatable fibrinolysis inhibitor (TAFI) into fibrin [9,35]. The main substrate for FXIII is fibrin, with only the α and γ chains participating in cross-linking activity [1]. There are several other proteins that FXIII cross-links: von Willebrand factor, factor V, vitronectin, vinculin, myosin, and actin [1]. Plasma FXIII binds to glycoprotein IIb/IIIa on platelets and α(v)β3 integrin on endothelial cells [36,37]. FXIII also plays an important function in wound healing and tissue repair by binding proteins fibronectin and collagen. The cross-linking of these proteins to fibrin enhances migration and proliferation of fibroblasts, which in turn stabilizes the extracellular matrix formed at the site of tissue injury [21,38].

Both plasma and platelet FXIII-A originate from the same gene, making them functionally indistinguishable [39,40]. Intracellular FXIII represents nearly 50% of total FXIII activity in the body [8]. Platelet FXIII-A is present in large concentrations in circulating platelets and appears to play an important role in the cytoskeletal remodeling associated with the activation of platelets [14,41]. Dale *et al.* showed that platelet factor XIII enhances thrombosis by cross-linking the primary amine serotonin to von Willebrand factor, factor V, and fibrinogen, which then localize to the platelet membrane via a serotonin receptor [42].

$FXIII\text{-}A_2$, produced in the placenta, plays an important role in early pregnancy. Low levels of maternal $FXIII\text{-}A_2$ can lead to poor formation of cytotrophoblastic shell and placental detachment. $FXIII\text{-}A_2$ is not critical for fertilization or early implantation but is necessary to minimize decidual bleeding starting at 5 weeks' gestation [43]. Impaired placental adhesion along with a tendency for hemorrhage in FXIII-deficient women likely lead to the increased risk of miscarriages and recurrent fetal loss reported.

Table 54.1 Factor XIII nomenclature.

Plasma FXIII	$FXIII\text{-}A_2B_2$
Cellular FXIII	$FXIII\text{-}A_2$
A subunit of FXIII	FXIII-A (monomer)
A subunit of FXIII	$FXIII\text{-}A_2$ (dimer)
B subunit of FXIII	FXIII-B (monomer)
B subunit of FXIII	$FXIII\text{-}B_2$ (dimer)
Inactive intermediate after thrombin cleavage	FXIIIa′
Thrombin + Ca^{2+}-activated FXIII	FXIIIa*

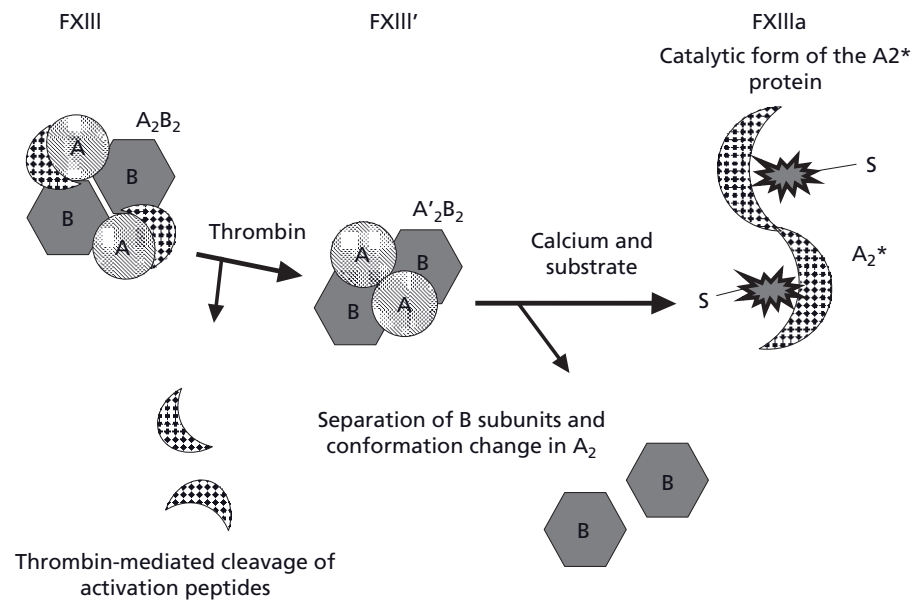

Figure 54.1 Activation of plasma factor XIII heterotetramer.

Molecular genetics

Since the first molecular mutation of FXIII deficiency was published in 1992, there have now been over 70 mutations reported [44,45]. The majority of the mutations for FXIII-A are owing to missense or nonsense mutations, occurring between exons 3 and 14 (Table 54.2). For a current listing of all subunit A or B mutations reported, please refer to the Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff website (http://www.hgmd.cf.ac.uk) and the Factor XIII Registry Database website (http://www.f13-database.de).

Table 54.2 Factor XIII-A missense/nonsense mutations.

Exon	Nucleotide change	Amino acid change	References
3	c.183C>A	Asn60Lys	Anwar *et al.* (1995)
3	c.232C>T	Arg77Cys	Duan *et al.* (2002)
3	c.233G>A	Arg77His	Peyvandi *et al.* (2004)
3	c.306G>A	Glu102Lys	Anwar (2002)
4	c.479T>G	Met159Arg	Schroeder *et al.* (2006)
4	c.514C>T	Arg171Stop	Standen and Bowen (1993)
5	c.631G>A	Gly210Arg	Vysokovsky *et al.* (2004)
5	c.646G>A	Gly215Arg	Schroeder *et al.* (2006)
6	c.707T>G	Leu235Arg	Birben *et al.* (2003)
6	c.728T>C	Met242Thr	Mikkola *et al.* (1994)
6	c.758G>T	Arg252Ile	Mikkola *et al.* (1996)
6	c.781C>T	Arg260Cys	Ichinose (1998)
6	c.782G>T	Arg260Leu	Vysokovsky *et al.* (2004)
6	c.782G>A	Arg260His	Kangsadalampai (1999)
6	c.788G>A	Gly262Glu	Onland *et al.* (2005)
7	c.851A>G	Tyr283Cys	Souri *et al.* (2001)
7	c.888C>G	Ser295Arg	Anwar *et al.* (2000)
7	c.949G>T	Val316Phe	Onland *et al.* 2005
7	c.956C>T	Ala318Val	Vysokovsky *et al.* (2004)
8	c.980G>A	Arg326Gln	Mikkola *et al.* (1996)
8	c.978C>T	Arg326Stop	Anwar (2005)
8	c.1064T>C	Leu354Pro	Anwar *et al.* (2001)
9	c.1128G>T	Trp375Cys	Schroeder *et al.* (2006)
9	c.1149G>T	Arg382Ser	Peyvandi *et al.* (2003)
9	c.1183C>T	Ala394Val	Izumi (1998)
9	c.1196C>A	Thr398Asn	Vysokovsky *et al.* (2004)
9	c.1201C>T	Gln400Stop	Kangsadalampai *et al.* (1996)
10	c.1226G>A	Arg408Gln	Anwar *et al.* 1995
10	c.1241C>T	Ser413Leu	Niya *et al.* (1999)
10	c.1241C>G	Ser413Trp	Duan *et al.* (2003)
10	c.1243G>T	Val414Phe	Aslam (1997)
10	c.1216G>A	Gly420Ser	Kangsadalampai *et al.* (2000)
11	c.1325C>A	Tyr441Stop	Anwar (1995)
11	c.1504G>A	Gly501Arg	Board (1993)
12	c.1496T>C	Leu498Pro	Mikkola *et al.* (1996)
12	c.1626C>G	Asn541Lys	Birben *et al.* (2002)
12	c.1687G>A	Gly562Arg	Takahashi *et al.* (1998)
14	c.1982T>C	Leu660Pro	Inbal *et al.* (1997)
14	c.1984C>T	Arg661Stop	Mikkola *et al.* (1994)
14	c.2003T>C	Leu667Pro	Aslam (1995)
15	c.2074G>A	Trp691Stop	Anwar (2005)
15	c.2150A>G	His716Arg	Schroeder *et al.* (2006)

www.f13-database.de/(rsg0bh45cx4x0v551wlea45)/content.aspx?menu=1,6

The vast majority of FXIII-deficient patients have mutations in the *F13A* gene, with more than 60 different types of mutations reported. Most of the mutations code for a single amino acid change (missense mutation), resulting in irregular folding and instability of the altered protein. Other types of mutations such as nonsense mutations, frame-shift mutations, and intronic mutations at splice sites leading to incorrect post-transcriptional processing of the mRNA have also been reported. Splice-site mutations have been reported to occur in introns 3, 5, 7, 8, 10, 11, and 14 [46]. To date, only four mutations have been described for the *F13B* gene, leading to the rarer form of the disease.

The FXIII-A gene (*F13A*) has been localized to chromosome 6p24–p25, spanning more than 160 kb, and has 15 exons separated by 14 introns encoding 731 amino acids (Figure 54.2). The FXIII-B gene (*F13B*) is located on chromosome 1q32–q32.1 and spans 28 kb in size with 12 exons interrupted by 11 introns encoding 641 amino acids [47,48] (Figure 54.3).

In addition to the numerous mutations reported in the FXIII-A gene, there are five common coding polymorphisms that have been identified: Val34Leu, Tyr204Phe, Pro564Leu, Val650Ile, and Glu651Gln. The most common single nucleotide polymorphism (SNP) is Val34Leu, occurring with an allele frequency of about 0.35 to 0.30 in the Caucasian population [49–51]. The nucleotide change from G to T does not result in FXIII deficiency, but does alter the function of the protein. Tyr204Phe variant occurs with the lowest frequency, at 0.01 to 0.03, and has been associated with an increased risk for recurrent miscarriages [52].

The Val34Leu variant has been most studied, with the amino acid substitution occurring in the activation peptide sequence, three amino acids upstream from the thrombin cleavage site. Studies show that activation of the Leu34 variant FXIII by thrombin proceeds more rapidly than the wild-type Val 34 [53]. Fibrin clots formed in the presence of Leu34 FXIII have thinner fibers, smaller pores, and altered permeation characteristics when compared with fibrin clots formed in the presence of Val34 variant [54]. Several studies support the protective role Val34Leu plays in myocardial infarctions and venous thrombosis [55–57]; however, there are reports of no association with or increased risk of thrombosis [58–61]. There is a correlation between geographic area and the Leu34 allele prevalence contributing to a difference in arterial thrombosis rates among different populations.

Two polymorphisms, Tyr204Phe and Pro564Leu, have been associated with an increased risk of hemorrhagic stroke in young women [62]. The other two polymorphisms, Glu651Gln and Val650Ile, have been studied the least.

The most common FXIII-B polymorphism occurs at codon 95 with replacement of histidine with arginine, with an allele

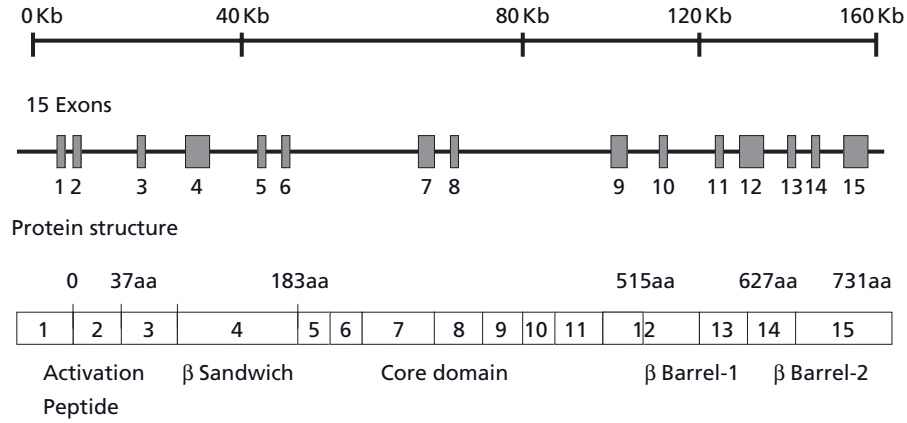

Figure 54.2 Factor XIII-A gene (*F13A*) and protein structure. *F13A* gene on chromosome 6 at bands p24–25: length 160 kb.

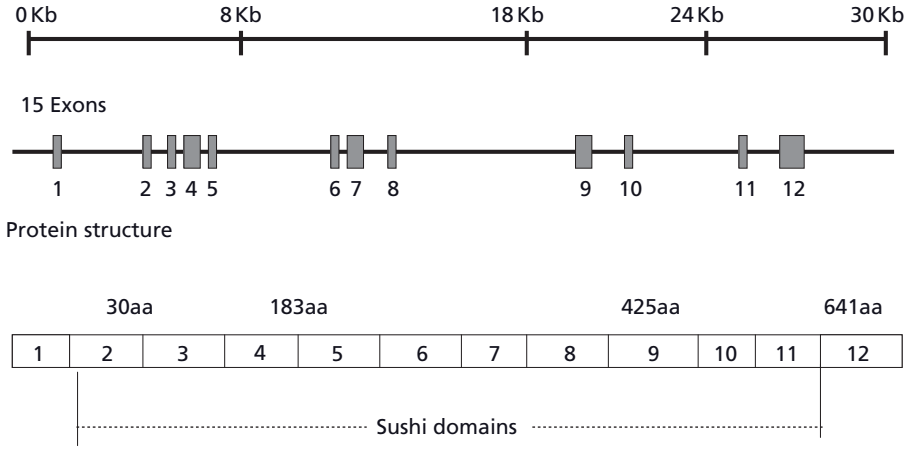

Figure 54.3 Factor XIII-B gene (*F13B*) and protein structure. The *F13B* gene is located on the long arm of chromosome 1 (q32–32.1). The 10 FXIIIB-subunit tandem repeats (known as Sushi domains) are encoded by a single exon 2–11.

frequency of 0.10 in the Caucasian population [21]. The current literature does not report any change of protein function with this specific nucleotide change in FXIII-B subunit.

As these examples of gene variants influencing phenotype suggest, there is a high level of heterogeneity observed in the clinical presentation among patients with FXIII deficiency. This heterogeneity represents the gamut of molecular variation of the FXIII gene: rare mutations to common polymorphisms.

Clinical presentation

The existence of FXIII was first discovered by Robbins in 1944 when he observed that insoluble fibrin interacted with calcium and an unknown "serum factor." Subsequent studies by Laki and Lorand confirmed its presence and role in coagulation, renaming it fibrin stabilizing factor (FSF). Sixteen years after the discovery of FSF, Duckert *et al.* described the first case report of a patient with FXIII deficiency, a young boy in Switzerland with a severe bleeding diathesis [63]. Recently, the molecular mutation of this first patient was described in the literature [64].

FXIII deficiency is a rare bleeding disorder with an incidence of 1 in 1–3 million individuals [65]. The inheritance pattern of FXIII deficiency is autosomal recessive, with the expected higher incidence among populations with consanguinity.

Clinical manifestations of FXIII deficiency include severe bleeding in the joints or soft tissue, spontaneous intracranial hemorrhages, poor wound healing, and spontaneous abortions. The international registry data recorded from 1993 to 2005 by Ivaskevicius *et al.* showed over half the patients (57%) presented with subcutaneous bleeding, followed closely by delayed umbilical cord bleeding (56%), a characteristic finding in these patients [66]. The incidence of intracranial hemorrhage has been reported to be 25–35%. This high incidence of life-threatening bleeding complications in FXIII-deficient patients distinguishes it from other types of bleeding disorders such as hemophilia A or B. This potentially fatal bleeding complication is the major reason the treatment plan for these patients is so aggressive. Postoperative bleeding 24–48 h later is commonly seen in FXIII-deficient patients because of normal clot formation, but early fibrinolysis because of weak cross-linking of fibrin. Ecchymoses and mucosal bleeding represent the more mild bleeding symptoms observed in these patients.

Patients with congenital homozygous FXIII deficiency present with severe bleeding symptoms and plasma FXIII levels of <1%. On the other hand, patients with heterozygous

mutations generally have a milder course without severe bleeding complications since their plasma levels are higher.

Homozygous congenital FXIII deficiency can be owing to defects in either FXIII-A genes (also known as type 2 defect) or FXIII-B genes (type 1 defect). The majority of patients with FXIII deficiency are lacking FXIII-A, leading to a greater tendency to bleed compared with patients missing FXIII-B. This clinical difference can be attributed to the preservation of intracellular FXIII-A in the latter case. Patients lacking FXIII-B will still have normal levels of FXIII-A in platelets to help stabilize clot formation.

Acquired FXIII deficiency has been reported in several diseases that lead to either impaired synthesis or increased consumption of the FXIII protein. Liver disease is the main cause of decreased FXIII production. Inflammatory diseases like Crohn's, ulcerative colitis, or sepsis, can cause local proteolytic degradation of FXIII [3]. Other diseases, such as systemic lupus erythematosus and leukemias, have also been associated with acquired FXIII deficiency. Inhibitors directed against FXIII protein can develop, resulting in acquired FXIII deficiency. Development of these autoantibody inhibitors frequently occur with the use of medications such as isoniazid, penicillin, and phenytoin [67]. Acquired FXIII deficiency can be treated with immune modulation therapy: steroids, intravenous immunoglobulin, anti-CD20 (rituximab), or plasmapheresis in rare instances.

Diagnosis

Since the transglutaminase enzyme acts in the last step of the clotting cascade after fibrin has already been formed, its activity is not reflected by routine coagulation assays. Standard screening tests, such as prothrombin time, activated partial thromboplastin time, fibrinogen level, platelet count, and bleeding time, are all normal in patients with FXIII deficiency. Clot formation is normal with FXIII deficiency, but the quality of the clot is weak because of lack of fibrin cross-linking. Qualitative testing for FXIII function includes clot solubility in 5 M urea and 1% monochloroacetic acid. Clots suspended in either solutions will lyse if plasma levels of FXIII are <1%. However, this test is only sensitive at extremely low levels of FXIII and will be normal if levels are above 1–3% [1]. Confirmatory quantitative testing can be accomplished using commercially available enzyme assays. Currently available is the Berichrom FXIII assay (Dade Behring AG, Marburg, Germany), which is based on a photometric method measuring the ammonia released in the first step of the transglutaminase reaction. The limitation of this test occurs at FXIII levels below 15% since the assay will report falsely elevated activity levels. The Pefakit FXIII Incorporation Assay (Pentapharm Ltd, Basel, Switzerland) or enzyme-linked immunosorbent assay (ELISA) measures FXIII cross-linking activity or FXIII subunit (A or B) antigens by antibodies, respectively. A new fluorometric assay developed by N-zyme BioTec (Darmstadt, Germany) is based on isopeptidase activity of FXIIIa described by Parameswaran *et al.* [68,69]. To date, only the Berichrom assay has been Food and Drug Administration (FDA)-approved for use within the USA.

Treatment

Once the diagnosis of FXIII deficiency is confirmed, prophylactic therapy with FXIII replacement is critical to prevent severe disability or even death because of bleeding complications. Traditionally, FXIII replacement came in the form of cryoprecipitate or fresh-frozen plasma. Cryoprecipitate can be given in doses of one bag per 10–20 kg every 3–4 weeks. Fresh-frozen plasma can be administered at 10 mL/kg every 4–6 weeks. Because of the long half-life of endogenous FXIII (ranging from 7 to 13 days), dosing frequency can be spaced out to 4–6 weeks for FXIII-containing products.

In the USA, the majority of FXIII-deficient patients are being managed by a plasma-derived pasteurized FXIII concentrate (Fibrogammin P; CSL Behring, Marburg, Germany). This product has been under clinical trials with more than 60 collaborating centers in the USA led by the Children's Hospital of Orange County (djn0@choc.org). The dosing for Fibrogammin P ranges from 10 to 35 U/kg every 4–6 weeks. Successful treatment with FXIII concentrate (Fibrogammin P) was confirmed by restoration of normal thromboelastography by Nugent in 2006 [70].

Recombinant FXIII-A_2 (Novo Nordisk; Bagsvaerd, Denmark) is a newer product currently undergoing phase III trials in the USA. This investigational drug is a homodimer (rFXIII-A_2) that binds to endogenous FXIII-B subunits circulating in the patients to form plasma FXIII-A_2B_2. Lovejoy *et al.* showed that his product was safe and effective as an alternative therapy for FXIII-deficient patients [71]. Studies for plasma-derived FXIII concentrates (Fibrogammin P) have not shown any development of neutralizing antibody production of the product over the past two decades. Ongoing studies with the more recently developed recombinant FXIII A2 will aid in determining the risk of inhibitor formation for this product.

Perinatal management of FXIII-deficient women varies according to the weeks of gestation. In general, coagulation factor levels tend to increase during gestation, with the exception of FXIII-A activity, which decreases during pregnancy. Since decidual bleeding begins early in gestation (week 5–6), FXIII replacement is critical to maintaining pregnancy. However, FXIII-A is not necessary for fertilization or early implantation. The half-life of FXIII-A concentrate ranges from 7 to 13 days during the nongestational period but progressively decreases to as low as 1.8 days in late pregnancy (32 weeks' gestation) [72,73]. Plasma levels of FXIII-A need to be sustained above 10% to prevent spontaneous abortion [74,75]. Recommended therapy for pregnancy at this time is with Fibrogammin P at a dosing of 250 IU every 7 days in early

pregnancy (less than 23 weeks' gestation) and increasing to 500 IU each 7 days later in pregnancy (greater than 23 weeks' gestation) [76,77]. During labor, a large booster dose of four vials (1000 IU) is recommended to reach plasma FXIII levels of 30% to avoid bleeding complications [78].

Prognosis

The risk for intracranial hemorrhage increases the morbidity and mortality of patients with FXIII deficiency. However, the prognosis is excellent with the current availability of plasma-derived products such as cryoprecipitate, fresh-frozen plasma, or FXIII concentrate. Lifelong therapy would be necessary to prevent bleeding complications, miscarriage, and especially life-threatening intracranial hemorrhage.

References

1 Anwar R, Miloszewski KJ. Factor XIII deficiency. *Br J Haematol* 1999; **107**: 468–84.
2 Lorand L, Losowsky MS, Miloszewski KJ. Human Factor XIII: fibrin-stabilizing factor. *Prog Hemost Thromb* 1980; **5**: 245–90.
3 Board PG, Losowsky MS, Miloszewski KJ. Factor XIII: inherited and acquired deficiency. *Blood Rev* 1993; **7**: 229–42.
4 Schwartz ML, Pizzo SV, Hill RL, *et al.* Human factor XIII from plasma and platelets: molecular weights, subunit structures, proteolytic activation, and cross-linking of fibrinogen and fibrin. *J Biol Chem* 1973; **248**: 1395–407.
5 Chung SI, Lewis MS, Folk JE. Relationships of the catalytic properties of human plasma and platelet transglutaminases (activated blood coagulation factor XIII) to their subunit structures. *J Biol Chem* 1974; **249**: 940–50.
6 Ashcroft AE, Grant PJ, Ariens RAS. A study of human coagulation factor XIII-subunit by electrospray ionization mass spectrometry. *Rapid Commun Mass Spectrom* 2000; **14**: 1607–11.
7 Muszbek L, Ariens RA, Ichinose A. On behalf of the ISTH SSC Subcommittee on Factor XIII. Factor XIII: recommended terms and abbreviations. *J Thromb Haemost* 2007; **5**: 181–3.
8 McDonagh J, McDonagh RP, Jr., Delage JM, *et al.* Factor XIII in human plasma and platelets. *J Clin Invest* 1969; **48**: 940–6.
9 Muszbek L, Adany R, Mikkola H. Novel aspect of blood coagulation Factor XIII. I. Structure, distribution, activation, and function. *Crit Rev Clin Lab Sci* 1996; **33**: 357–421.
10 Nagy B, Jr., Simon Z, Bagoly Z, *et al.* Binding of plasma factor XIII to thrombin-receptor activated human platelets. *Thromb Haemost* 2009; **102**: 83–9.
11 Henricksson P, Becker S, Lynch G, *et al.* Identification of intracellular factor XIII in human monocytes and macrophages. *J Clin Invest* 1985; **76**: 528–34.
12 Wolpl A, Lattke H, Board PG, *et al.* Coagulation factor XIII A and B subunits in bone marrow and liver transplantation. *Transplantation* 1987; **43**: 151–3.
13 Poon MC, Russell JA, Low S, *et al.* Hematopoietic origin of factor XIII A subunits in platelets, monocytes, and plasma. Evidence from bone marrow transplantation studies. *J Clin Invest* 1989; **84**: 787–92.
14 Jayo A, Conde I, Lastres P, *et al.* New insights into the expression and role of platelet factor XIII-A. *J Thromb Haemost* 2009; **7**: 1184–91.
15 Yorifuji H, Anderson K, Lynch GW, *et al.* B protein of factor XIII: differentiation between free B and complexed B. *Blood* 1988; **72**: 1645–50.
16 Nagy JA, Henriksson P, McDonagh J. Biosyntheisis of factor XIII B subunit by human hepatoma cell lines. *Blood* 1986; **68**: 1272–9.
17 Siebenlist KR, Meh DA, Mosesson MW. Plasma factor XIII binds specifically to fibrinogen molecules containing gamma chains. *Biochemistry* 1996; **35**: 10448–53.
18 Mosesson MW. Fibrinogen gamma chain functions. *J Thromb Haemost* 2003; **1**: 231–8.
19 Yee VC, Pedersen LC, Le Trong, *et al.* Three-dimensional structure of a transglutaminase: human blood coagulation factor XIII. *Proc Natl Acad Sci USA* 1994; **91**: 7296–300.
20 Weiss MS, Metzner HJ, Hilgenfeld R. Two non-proline cis peptide bonds may be important for factor XIII function. *FEBS Lett* 1998; **423**: 291–6.
21 Ariens RAS, Lai TS, Weisel JW, *et al.* Role of factor XIII in fibrin clot formation and effects of genetic polymorphisms. *Blood* 2002; **100**: 743–54.
22 Ichinose A, McMullen BA, Fjuikawa K, *et al.* Amino acid sequence of the b subunit of human factor XIII, a protein composed of ten repetitive segments. *Biochemistry* 1986; **25**: 4633–8.
23 Souri M, Kaetsu H, Ichinose A. Sushi domains in the B subunit of factor XIII responsible for oligomer assembly. *Biochemistry* 2008; **47**: 8656–64.
24 Mikuni, Y, Iwanga S, Konishi KA. Peptide released from plasma fibrin stabilizing factor in the conversion to the active enzyme by thrombin. *Biochem Biophys Res Commun* 1973; **54**: 1393–402.
25 Nakamura S, Iwanga S, Suzuki T, *et al.* Amino acid sequence of the peptide released from bovine factor XIII following activation by thrombin. *Biochem Biophys Res Commun* 1974; **58**: 250–6.
26 Takgai T, Doolittle RF. Amino acid sequence studies on factor XIII and the peptide released during its activation by thrombin. *Biochemistry* 1974; **13**: 750–6.
27 Lorand L, Konishi K. Activation of the fibrin stabilizing factor of plasma by thrombin. *Arch Biochem Biophys* 1964; **105**: 58–67.
28 Pisano JJ, Finlayson JS, Peyton MP. Crosslink in fibrin polymerized by factor XIII:e-(gamma-glutamyl) lysine. *Science* 1968; **160**: 892–3.
29 Matacic S, Loewy AG. The identification of isopeptide crosslink in insoluble fibrin. *Biochem Biophys Res Com* 1968; **30**: 356–62.
30 Greenberg CS, Miraglia CC, Rickles FR, *et al.* Cleavage of blood coagulation factor XIII and fibrinogen by thrombin during in vitro clotting. *J Clin Invest* 1985; **75**: 1463–70.
31 Kanaide H, Shainoff JR. Cross-linking of fibrinogen and fibrin by fibrin-stabilizing factor (Factor XIIIa). *J Lab Clin Med* 1975; **85**: 574–97.
32 Mosesson MW, Siebenlist KR, Hainfeld JF, *et al.* The covalent structure of factor XIIIa crosslinked fibrinogen fibrils. *J Str Biol* 1995; **115**: 88–101.
33 Greenberg GS, Achyuthan KE, Fenton JW. Factor XIIIa formation promoted by complexing of alpha-thrombin, fibrin, and plasma factor XIII. *Blood* 1987; **69**: 867–71.
34 Lorand L, Gray AJ, Brown K. Dissociation of the subunit structure of fibrin stabilizing factor during activation of the zymogen. *Biochem Bophys Res Com* 1974; **56**: 914–22.
35 Lorand L. Factor XIII: Structure, activation, and interactions with fibrinogen and fibrin. *Ann NY Acad Sci* 2001; **936**: 291–311.
36 Cox AD, Devine DV. Factor XIIIa binding to activated platelets is mediated through activation of glycoprotein IIb-IIIa. *Blood* 1994; **83**: 1006–16.

37 Dardik R, Shenkman B, Tamarin I, *et al.* Factor XIII mediates adhesion of platelets to endothelial cells through alpha(v)beta(3) and glycoprotein IIb/IIIa integrins. *Thromb Res* 2002; **105**: 317–23.

38 Schroeder V, Durrer D, Meili E, *et al.* Congenital factor XIII deficiency in Switzerland: from the worldwide first case in 1960 to its molecular characterisation in 2005. *Swiss Med Wkly* 2007; **137**: 272–8.

39 Board PG. Genetic polymorphism of the A subunit of human coagulation factor XIII. *Am J Hum Genet* 1979; **31**: 116–24.

40 Chung SI. Comparative studies on tissue transglutaminase and factor XIII. *Ann NY Acad Sci* 1972; **202**: 240–55.

41 Adany R, Bardos H. Factor XIII subunit A as an intracellular transglutaminase. *Cell Mol Life Sci* 2003; **60**: 1049–60.

42 Dale GL, Friese P, Batar P, *et al.* Stimulated platelets use serotonin to enhance their retention of procoagulant proteins on the cell surface. *Nature* 2002; **415**: 175–9.

43 Asahina T, Kobayashi T, Takeuchi K, *et al.* Congenital blood coagulation factor XIII deficiency and successful deliveries: a review of the literature. *Obstet Gynecol Surv* 2007; **62**: 255–60.

44 Kamura T, Okamura T, Murakawa M, *et al.* Deficiency of coagulation factor XIII A subunit caused by the dinucleotide deletion at the 5′ end of exon III. *J Clin Invest* 1992; **90**: 315–19.

45 Board P, Coggan M, Miloszewski K. Identification of a point mutation in factor XIII A subunit deficiency. *Blood* 1992; **80**: 937–41.

46 Schroeder V, Durrer D, Mell E, *et al.* Congenital factor XIII deficiency in Switzerland. *Swiss Med Wkly* 2007; **137**: 272–8.

47 Bottenus RE, Ichinose A, Davie EW. Nucleotide sequence of the gene for the b subunit of human factor XIII. *Biochemistry* 1990; **29**: 11195–209.

48 Webb GC, Coggan M, Ichinose A, *et al.* Localization of the coagulation factor XIIIB subunit gene (F13B) to chromosome bands 1q31–32.1. and restriction fragment length polymorphism at the locus. *Hum Genet* 1989; **81**: 157–60.

49 Kohler HP, Stickland MH, Ossei-Gerning N, *et al.* Association of a common polymorphism in the factor XIII gene with myocardial infraction. *Thromb Haemost* 1998; **79**: 8–13.

50 McCormack LJ, Kain K, Catto AJ, *et al.* Prevalence of FXIII V34L in populations with different cardiovascular risk. *Thromb Haemost* 1998; **80**: 601–3.

51 Attie-Castro FA, Zago MA, Lavinha J, *et al.* Ethnic heterogeneity of the factor XIII Val34Leu polymorphism. *Thromb Haemost* 2000; **84**: 601–3.

52 Anwar R, Gallivan L, Edmonds SD, *et al.* Genotype/phenotype correlations for coagulation factor XIII: specific normal polymorphisms are associated with high or low factor XIII specific activity. *Blood* 1999; **93**: 897–905.

53 Wartiovaara U, Mikkola H, Szoke G, *et al.* Effect of Val34Leu polymorphism on the activation of the coagulation factor XIII-A. *Thromb Haemost* 2000; **84**: 595–600.

54 Ariens RAS, Philippou H, Nagaswami C, *et al.* The factor XIII V34L polymorphism accelerates thrombin activation of factor XIII and affects cross-linked fibrin structure. *Blood* 2000; **96**: 988–95.

55 Hancer VS, Diz-Kucukkaya R, Bilge AK, *et al.* The association between factor XIII Val34Leu polymorphism and early myocardial infarction. *Circ J* 2006; **70**: 239–42.

56 Wells PS, Anderson JL, Scarvelis DK, *et al.* Factor XIII Val34Leu variant is protective against venous thromboembolism: a HuGE review and meta-analysis. *Am J Epidemiol* 2006; **164**: 101–9.

57 Catto AJ, Kohler HP, Coore J, *et al.* Association of a common polymorphism in the factor XIII gene with venous thrombosis. *Blood* 1999; **93**: 906–8.

58 Franco RF, Reitsma PH, Lourenco D, *et al.* Factor XIII Val34Leu is a genetic factor involved in the etiology of venous thrombosis. *Thromb Haemost* 1999; **81**: 676–9.

59 Aleksic N, Ahn C, Wang YW, *et al.* Factor XIII Val34Leu polymorphism does not predict risk of coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. *Arterioscler Thromb Vasc Biol* 2002; **22**: 348–52.

60 Balogh I, Szoke G, Karpati L, *et al.* Val34Leu polymorphism of plasma factor XIII: biochemistry and epidemiology in familial Thrombophilia. *Blood* 2000; **96**: 2479–86.

61 Margaglione M, Bossone A, Brancaccio V, *et al.* Factor XIII Val34Leu polymorphism and risk of deep vein thrombosis. *Thromb Haemost* 2000; **84**: 1118–19.

62 Reiner AP, Schwartz SM, Frank MB, *et al.* Polymorphisms of coagulation factor XIII subunit A and risk of nonfatal hemorrhagic stroke in young white women. *Stroke* 2001; **32**: 2580–6.

63 Duckert F, Jung E, Shmerling DH. A hitherto undescribed congenital haemorrhagic diathesis probably due to fibrin stabilizing factor deficiency. *Thromb Diath Haemorrh* 1960; **5**: 179–86.

64 Schroeder V, Meili E, Cung T, *et al.* Characterization of six novel A-subunit mutations leading to congenital factor XIII deficiency and molecular analysis of the first diagnosed patient with this rare bleeding disorder. *Thromb Haemost* 2006; **95**: 77–84.

65 Ichinose A. Physiopathology and regulation of factor XIII. *Thromb Haemost* 2001; **86**: 57–65.

66 Ivaskevicius V, Seitz R, Kohler HP, *et al.* International registry on factor XIII deficiency: a basis formed mostly on European data. *Thromb Haemost* 2007; **97**: 914–21.

67 Tosetto A, Castaman G, Rodeghiero F. Acquired plasma factor XIII deficiency. *Haemotologica* 1993; **78**: 5–10.

68 Oertel K, Hunfeld A, Specker E, *et al.* A highly sensitive fluorometric assay for determination of human coagulation factor XIII in plasma. *Anal Biochem* 2007; **367**: 152–8.

69 Parameswaran KN, Cheng XF, Chen EC, *et al.* Hydrolysis of γ:ε isopeptides by cytosolic transglutaminases and by coagulation factor XIIIa. *J Biol Chem* 1997; **272**: 10311–17.

70 Nugent DJ. Prophylaxis in rare coagulation disorders—factor XIII deficiency. *Thromb Res* 2006; **118**: S23–8.

71 Lovejoy AE, Reynolds TC, Visich JE, *et al.* Safety and pharmacokinetics of recombinant factor XIII-A2 administration in patients with congenital factor XIII deficiency. *Blood* 2006; **108**: 57–62.

72 Rodegheiro F, Fastaman GC, Di Bona E, *et al.* Successful pregnancy in a woman with congenital factor XIII deficiency treated substitutive therapy. *Blut* 1987; **55**: 45–8.

73 Kadir R, Chi C, Bolton-Maggs P. Pregnancy and rare bleeding disorders. *Haemophilia* 2009; **15**: 990–1005.

74 Kobayshi T, Terao T, Kojima T, Takamatsu J, Kamiya T, Saito H. Congenital factor XIII deficiency with treatment of factor XIII concentrate and normal delivery. *Gynecol Obstet Invest* 1990; **29**: 235–8.

75 Asahina T, Kobayshi T, Okada Y. Studies on the role of adhesive proteins in maintaining pregnancy. *Horm Res* 1998; **50**(Suppl. 2): 37–45.

76 Ichinose A, Asahina T, Kobayashi T. Congenital blood coagulation factor XIII deficiency and perinatal management. *Curr Drug Targets* 2005; **6**: 541–9.

77 Asahina T, Kobayashi T, Takeuchi K, *et al.* Congenital blood coagulation factor XIII deficiency and successful deliveries: a review of the literature. *Obstet Gynecol Surv* 2007; **62**: 255–60.

78 Inbal A, Musbek L. Coagulation factor deficiencies and pregnancy loss. *Semin Thromb Hemost* 2003; **29**: 171–4.

55 Fibrinogen

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Introduction

Fibrinogen is a soluble 340-kDa dimeric plasma glycoprotein. Its principal importance lies in its conversion into the insoluble fibrin clot by the action of thrombin. However, it also has important interactions with other adhesion molecules, platelets, endothelial cells, and cells involved in the inflammatory response. Where appropriate, the nomenclature used here follows published guidelines although numbering of amino acids refers to the mature processed molecule pending further agreement [1].

Fibrinogen structure

Each half of the fibrinogen molecule is composed of three polypeptide chains, designated Aα, Bβ, and γ. The properties of each chain are listed in Table 55.1.

The amino-terminals of the three pairs of polypeptide chains are held together by disulfide bridges (two γ-γ and one α-α) to form the globular E region (Plate 55.1). All of the total 58 cysteine residues in each six-chained molecule are incorporated into 29 inter- and intrachain disulfide bridges. From the central E region the two sets of three chains extend out in an antiparallel fashion in a coiled coil to the D region which comprises the somewhat separate globular carboxy-termini of the γ- and β-chains. From this region the α chain extends back in a structure called the Cα region with its carboxy-terminus resting adjacent to the E region (Plate 55.1). The available crystal structure of human fibrinogen has been obtained in a piecemeal fashion and construction of models has been aided by studies in other species [2].

A variant γ chain, denoted γ′, is produced by a variation in mRNA splicing which results in the replacement of aa residues 408–411 by a novel 20 aa residues terminating at residue 427. In the process a platelet-binding site is lost and high-affinity binding sites for factor XIII and thrombin gained. The γ′ variant constitutes approximately 10% of plasma fibrinogen. A less common (<2%) α chain variant "αE" is also produced by splice variation and has an additional 236 carboxy-terminal amino acids.

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Genetics and regulation of synthesis

The sequences of the three fibrinogen (FG) chains exhibit a high degree of homology, suggesting that they have arisen by duplication events. This conclusion is supported by similarities in the intron–exon structure of the three genes *FGA*, *FGB*, and *FGG* and their genomic position in a contiguous cluster on chromosome 4q31.3, with Bβ in the opposite transcriptional orientation to Aα and γ. Transcription of the three genes is highly coordinated, but at the protein level production of Bβ chain seems to be the limiting factor in humans. Studies from liver transplant patients indicate that the liver is the only major site of fibrinogen synthesis in man [3], producing 1.7–5.0 g per day; sufficient to replace metabolized/utilized fibrinogen in the steady-state condition. The plasma half-life is 3–5 days. Fibrinogen gene mRNA transcripts have been identified in other tissues such as epithelial and carcinoma cells, which may be important locally [4]. Synthesis of fibrinogen is upregulated by interleukin 6 (IL-6) as part of the acute phase reaction but IL-1β and transforming growth factor (TGF)-β have an opposing effect. Several polymorphisms of the fibrinogen genes have been described and most interest has focused on the –455G/A dimorphism in the *FGB* gene. This is estimated to account for 1–5% of the population variation in fibrinogen levels. The presence of the A allele was associated with a 0.15 g/L rise in fibrinogen level [5]. Other studies have only detected this effect in smokers or those with coronary artery disease, suggesting an environmentally influenced dynamic expression of the allele [6,7].

Plasma fibrinogen is an heterogeneous pool resulting from differential splicing of mRNA transcripts (Table 55.1) and from partial cleavage in plasma by thrombin and plasmin. Fibrinogen is also found in platelets but this is derived from $\alpha_{IIb}\beta_3$ (glycoprotein IIbIIIa)-mediated endocytosis of plasma fibrinogen which is then stored in α granules, rather than synthesized by megakaryocytes. Thus, fibrinogen mRNA is not detected in megakaryocytes [8] and platelet fibrinogen is absent in severe forms of Glanzmann thrombasthenia [9,10].

Fibrin clot formation

Conversion of fibrinogen into the insoluble fibrin clot can be considered to take place in three stages:

1 thrombin cleavage of fibrinopeptides A and B;

Table 55.1 Characteristics of the fibrinogen chains

Chain	Aα	Bβ	γ
Amino acid residues	610	461	411
Molecular weight	66 500	52 000	46 500
Variants (% total)	AαE + 296aa (2%)		γ′ + 408 – 427 (10%)
Thrombin cleavage	R16–17G	R14–15G	—
Cystine residues	8	11	10
Factor XIII cross-link residues (Gln acceptor, Lys donor)	Gln: 221, 237, 328, 366 Lys: 208, 219, 224, 418, 427, 429, 446, 448, 508, 539, 556, 580, 583, 601, 606	None	Gln: 398, 399 Lys: 406
Glycosylation sites	(αE) Asn 667	Asn 364	Asn 52
$\alpha_{IIb}\beta_3$ binding RGDF motif	95–98		
$\alpha_{IIb}\beta_3$ binding RGDS motif	572–575		
$\alpha_{IIb}\beta_3$ binding Dodecapeptide			400–411
$\alpha_{IIb}\beta_3$ and $\alpha_5\beta_1$ binding site			370–383
$\alpha_v\beta_3$ on endothelial cells			346–358

2 assembly of insoluble fibrin monomers into protofibrils and fibers; and
3 cross-linking by factor XIII.

The initiating event in fibrin formation is the cleavage of the Aα Arg16–Gly17 bond by thrombin, releasing fibrinopeptide A (FpA). This exposes an "A" site (knob) in the E region which can then bind to a pre-existing "a" site (hole) on the γ chain of another molecule's D region. Thus begins a staggered assembly of half-overlapping fibrin monomers which extends to form a double-stranded protofibril (Plate 55.2). At this stage, FXIII activation by thrombin has already occurred and cross-linking has begun (see below).

Cleavage of fibrinopeptide B (FpB) follows that of FpA; however, this sequential release of FpA and then FpB reflects the differing affinity of thrombin for these two substrates rather than a change in conformation after FpA release [11]. Fibrin polymerization facilitates FpB cleavage but it remains incomplete with only 33% of the potential FpB being released. FpB release exposes a "B" site in the E region which binds to a corresponding "b" site on the carboxy-terminus of a β chain in an adjacent molecule. This facilitates lateral assembly of thick fibers. FpB cleavage also releases the Cα region from its noncovalent association with the E region, freeing it to participate in lateral interactions with other Cα regions [12]. Nonetheless, FpA release appears sufficient for the entire process of clot formation, although it is enhanced by FpB removal and mutations of the FpB cleavage site result in prolonged clotting times and reduced functional fibrinogen assay results. FpB cleavage alone (e.g., by Venzyme [13]) is not sufficient to induce fibrin clot formation under normal conditions.

Lateral association of fibrils leads to formation of thick fiber bundles. Convergence of two or three double-stranded fibrils results in tetra- and tri-molecular branch points, effectively joining the nascent fibers and producing a complex three-dimensional network [14].

Formation of fibrin also accelerates the formation of activated factor XIII (FXIII). In plasma, fibrinogen functions as a carrier protein for the a2b2 FXIII tetramer which appears to bind specifically to the γ′ chains. Fibrin polymer formation accelerates FXIII cleavage but the a subunit is not active until the activation peptide has been released and has dissociated from the b subunit. One or both of these dissociation events are facilitated by binding of activated FXIII to the γ′ region of fibrin. The active a subunit released then binds to fibrin in the Aα 242–424 region and leaves behind a vacant thrombin-binding site. Thus, fibrin formed from γ′ containing fibrinogen is more extensively cross-linked and higher γ′ levels are a risk factor for coronary heart disease [15]. Platelet FXIII comprises the a units only and is not dependent on fibrin for activation.

FXIII-mediated cross-linking begins by joining D regions in an end-to-end or longitudinal fashion via reciprocal γGln398–γLys406 links [16]. Side-to-side links between γ chains then follow. Cross-linking between α chains occurs more slowly but at multiple sites (Table 55.1). "α" chain cross-linking is important in blocking plasmin access to the coiled-coil regions linking D and E regions and thus preventing breakdown of the fibrin clot. Finally, some cross-linking between α and γ chains takes place [17].

At the same time, fibrin formation exposes binding sites for tPA and plasminogen. High-affinity sites for both tPA and plasminogen are exposed in the Aα392–610 segment (Cα) and lower affinity sites on Aα 148–160 and γ 312–324 (D region). Adjacent binding of tPa and plasminogen greatly reduces the K_m and increases the V_{max} for the activation reaction [18]. The plasmin thus activated then cleaves fibrin, exposing further carboxy-terminal lysine residues of Cα, allowing more plasminogen and tPA to bind, accelerating and targeting the fibrinolytic system. This process is now known to be in competition with the activity of TAFI (thrombin activatable fibrinolysis inhibitor), which selectively removes these lysine residues and thus limits fibrinolysis. FXIII also cross-links antiplasmin to Lys 303 in the α chain whilst retaining its antiplasmin activity. It has recently been shown that this occurs extensively in plasma so that approximately 1.2–1.8 moles of antiplasmin are bound per mole of fibrinogen [19].

Fibrinogen interaction with other cells

In addition to its self-associating properties, fibrin also has other important interactions. At least four integrin-binding motifs have been identified in fibrinogen, not all of which contain the classic RGD sequence (Table 55.1). The γ-terminal sequence is involved in platelet adherence and aggregation whereas the two α chain RGD sequences are important in promoting clot retraction [20]. These sequences mediate binding to platelet $\alpha_{IIb}\beta_3$ to effect platelet retraction but also to leukocyte $\alpha_M\beta_2$ and to endothelial cell $\alpha_V\beta_3$. Mice with fibrinogen lacking the terminal γ chain sequence had a severe bleeding tendency [20]. Fibrinogen binding to endothelial cell integrins is mediated by the Aα RGD sequence at 572–574 [21] and by the γ chain 346–358 [22]. However, fibrin-specific binding to endothelial cells is mediated by the β15–42 region exposed by thrombin cleavage. Fibrin or fibrin peptide interaction with endothelial cells results in numerous changes including release of Weibel–Palade bodies, prostacyclin, and tPA release and monolayer disruption [23–25]. Finally, fibrinogen is important in mediating adherence and migration of activated monocytes and neutrophils through endothelium [26].

Fibrinogen is also able to bind several cytokines, in particular vascular endothelial growth factor, FGF-2, and IL-1β. This is postulated to concentrate the factors at the site of vascular injury and promote wound healing. Loss of this function may contribute to the phenotype seen in afibrinogenemic mice as well as in humans [27].

Measuring fibrinogen

An international standard for fibrinogen assays has been prepared [28]. The normal plasma concentration of fibrinogen is approximately 1.7–4.0 g/L when measured by clotting assays and slightly higher (2.5–6.0 g/L) by immunoassay. Local normal ranges should be determined. The ability to detect abnormalities of fibrinogen depends critically on the assay methods used and guidelines for fibrinogen assays have been published in the UK [29]. A deficiency or abnormality of fibrinogen may be first suspected from prolongation of clotting times. In afibrinogenemia all the clotting times will be prolonged but in less marked deficiency or the presence of a dysfibrinogenemia, it may be only the thrombin time which is prolonged. Dempfle reported that the clotting times became prolonged only when the fibrinogen fell below 1 g/L, but this is likely to vary with other circumstantial factors and the reagents used [30].

Although the international reference method for fibrinogen measurement has traditionally been by the total clottable fibrinogen method, this is not practical for routine laboratory use. In hospital practice, fibrinogen is usually assayed by a functional (clotting) assay, either that of Clauss or as a measure derived from the optical changes occurring during the prothrombin time performed on an automated analyzer (derived fibrinogen: PT-Fg). In the Clauss assay [31] a high concentration of thrombin is used to clot a diluted aliquot of patient plasma. Typically one volume of a 100 NIH U/mL thrombin solution is added to two volumes of plasma to give a final concentration of approximately 33 NIH U/mL. The clotting time is then compared with a standard curve prepared using dilutions of a reference plasma. When automated, the assay is quite reproducible (coefficient of variation 3–9%) and the combination of dilution and high thrombin concentration makes it relatively insensitive to the effect of heparin. It is, however, sensitive to high concentrations of fibrin(ogen) degradation products and some kits contain agents to overcome these effects. It has recently been suggested that a reliable measure of fibrinogen concentration may be obtained using rotational thromboelastography [32].

The PT-Fg generally results in a slightly higher estimation of fibrinogen than the Clauss. This is particularly so when coagulation is deranged for some reason or an abnormal fibrinogen is present. The PT-Fg is therefore not recommended [29], although it remains in widespread use as a result of its low cost and convenience. Fibrinogen can also be assayed using immunologic techniques, but this requires the use of monoclonal antibodies to avoid interference from degraded and partially degraded fibrinogen fragments. The performance of different fibrinogen assays has been reviewed [33].

When a low fibrinogen is determined by clotting assay then the presence of a dys- or hypo- fibrinogen can be resolved by performing a physicochemical or immunologic assay. In practice, a simple (gravimetric) clot weight determination is usually sufficient to demonstrate the discrepancy between function and protein characteristic of a dysfibrinogenemia [33].

Afibrinogenemia

The complete absence of fibrinogen, afibrinogenemia, is accompanied by a clinical syndrome that is surprisingly mild and generally less severe than hemophilia (Table 55.2) [34]. An early and characteristic feature is umbilical cord bleeding—present in 85% of cases [34]. Thereafter, muscle bleeds and hemarthroses are relatively frequent but rarely result in disability. Unlike hemophilia, epistaxis is also common (72%) and spontaneous splenic rupture is reported. Bleeding is sometimes followed by poor wound healing [34]. In mice rendered afibrinogenemic by disruption of the *FGA* gene, all three chains are absent from plasma and a similar phenotype is seen: bleeding begins shortly after birth in approximately 30% but is not usually severe and the mice survive into maturity [35].

In affected women, menorrhagia is common and recurrent early abortion is seen, presumed to reflect poor implantation as also observed in the fibrinogen-deficient mice. Afibrinogenemic mice are still capable of developing atheromatous plaques, although murine plaques are not as complex as those in humans [36].

Table 55.2 Relative frequency of bleeding symptoms in 55 patients with afibrinogenemia compared with 100 patients with severe hemophilia A (factor VIII 1%)

Symptom	In afibrinogenemia	In hemophilia A
Umbilical cord bleeding	45/55 (85%)	0
Central nervous system bleeding	3/55 (10%)	4/100 (4%)
Hemarthrosis	30/55 (54%)	86/100 (86%)
Muscle hematoma	40/55 (72%)	93/100 (93%)
Gastrointestinal bleeding	0	10/100 (10%)
Urinary tract bleeding	0	12/100 (12%)
Epistaxis	40/55 (72%)	50/100 (50%)
Menorrhagia	14/20 (70%)	Not applicable
Oral cavity bleeding	40/55 (72%)	55/100 (55%)
Postoperative bleeding	23/55 (40%)	36/100 (36%)
Thrombotic symptoms	2/55 (4%)	0

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Remarkably, spontaneous thrombosis (venous and arterial) has been reported in patients with afibrinogenemia. A plausible explanation is that an increase in free thrombin results from the loss of the thrombin-binding capacity of fibrin and that this results in excessive platelet activation [37].

Genetics and molecular biology

Inheritance of afibrinogenemia is autosomal recessive and the disorder is thus more common in populations favoring consanguineous marriages. The estimated frequency in Europe is 1 in 10^6. Obligate heterozygotes have plasma fibrinogen levels of approximately half the normal level, but are asymptomatic. The majority of cases appear to arise from mutations in the *FGA* gene; in particular, a recurrent 11-kb deletion probably results from a nonhomologous recombination mediated by 7-bp direct repeats. Haplotype analysis suggests that the mutation has arisen independently on several occasions [38]. Indeed, the majority of null mutations are in the *FGA* gene and the most common recurrent mutation occurs at the donor splice site of *FGA* intron 4 [39]. A number of other truncating mutations in *FGA* as well as *FGB* and *FGG* are also described (reviewed in refs 40 and 41). Failure to produce any of the individual peptide chains results in a virtually complete absence of the mature protein. A small number of missense mutations also cause afibrinogenemia as a result of intracellular retention of the abnormal protein [42].

Therapy

The basis of therapy is replacement of fibrinogen. Historically, the principal source has been cryoprecipitate, which contains approximately 1.5 g fibrinogen per unit. Plasma-derived, virally inactivated concentrates are available and are the recommended choice but are not uniformly licensed. When concentrate is not available, UK-BCSH guidelines recommend virally inactivated plasma over cryoprecipitate if volume is not a limiting factor [43]. Methylene blue treatment of plasma is reported to result in a 40% loss of fibrinogen [43] but fibrinogen content of solvent-detergent-treated plasma is normal [44]. The half-life of infused fibrinogen concentrate was similar in two studies, at approximately 3–5 days. However, the recovery showed considerable variation: in the study by Negrier *et al.* a dose of 0.06 g/kg produced a rise of 1.39 g/L, whereas the data from Kreuz *et al.* suggest a similar dose would produce a rise of 0.84 g/L [45,46].

A level of 1 g/L is regarded as sufficiently hemostatic to arrest bleeding (20–40 mg/kg of fibrinogen concentrate). Bleeding problems may be sufficient to warrant prophylactic treatment and the long half-life of fibrinogen allows prophylactic infusions of fibrinogen concentrate or cryoprecipitate to be given weekly. In one report a dose of 100 mg/kg/week was sufficient to maintain a trough level of 0.5 g/L, which prevented recurrence of hemorrhage [47]. However, in the context of primary prophylaxis, even lower levels may be effective in preventing bleeding and 2–4 weekly infusions have been reported as successful [48]. A survey of hematologists reported a considerable diversity of practice: prophylactic doses ranged from 18 to 120 mg/kg (median 53 mg/kg) and most patients (59%) were treated weekly (remainder 2- to 4-weekly). However, the survey also revealed that prophylaxis completely prevented bleeds in only 9 of 19 patients [49]. Replacement therapy is not usually complicated by the development of antifibrinogen antibodies, but these have been reported [50]. Menorrhagia may be controlled by use of the combined oral contraceptive pill, and adjunctive therapy with tranexamic acid has also been suggested.

Prophylactic therapy appears necessary for successful completion of pregnancy: fibrinogen replacement must be begun before 5 weeks' gestation to prevent abortion and maintained at >1.0 g/L throughout the pregnancy. Fibrinogen consumption increases markedly as gestation progresses, and the amount and frequency of fibrinogen infusion must be increased accordingly. A level of >1.5 g/L is recommended for delivery [51].

Hypofibrinogenemia may be used to describe patients with incomplete deficiency of fibrinogen and a corresponding intermediate phenotype. Some of these will be heterozygotes for null mutations and are generally asymptomatic.

Dysfibrinogenemia

Dysfibrinogens are usually inherited in an autosomal dominant fashion but a few cases are found as recessives with asymptomatic heterozygotes. Dysfibrinogen generally arises from missense mutations in any of the three *FG* genes, but small deletions or insertions may also be responsible and the clinical phenotype is correspondingly diverse. A database of mutations responsible is available at http://www.geht.org/databaseang/fibrinogen/. The diagnosis is suspected from

prolonged coagulation times, especially the thrombin time, with low fibrinogen by functional assay. Determination of a discrepantly high fibrinogen protein concentration confirms the presence of a dysfibrinogen. Rare examples where the clotting times are shortened or where the functional and immunologic assays are concordant have been described.

The majority of dysfibrinogens are found incidentally and have no associated phenotype (55%), whereas 20% are associated with thrombosis and 25% with hemorrhage. A few cases have been reported with both hemorrhagic and thrombotic problems (e.g., Marburg, Bethesda III, and Baltimore I) and in some cases the phenotype may have been modified by other traits such as factor V Leiden [52]. It is not possible to predict the phenotype from the standard laboratory tests and so sequencing of the gene has been recommended to allow comparison with other published cases [52]. Studies of dysfibrinogens have been very informative in unravelling the structure–function relationships of fibrinogen [53].

Dysfibrinogens associated with thrombosis

Although usually detected as a result of a prolonged thrombin clotting time, a dysfibrinogen is reported in approximately 0.8% of patients with thrombosis; rarer than the anticoagulant deficiencies, but, unlike them, associated with both venous and arterial events, although venous are more common. The rarity of each individual dysfibrinogen makes it difficult to be certain of a causal relationship underlying the association, but a survey conducted by the ISTH in 1995 [54] concluded it was genuine: an increased incidence of thrombosis was found in affected relatives of the dysfibrinogenemia proband but not in the 88 unaffected relatives. As with other thrombophilic traits, women affected with dysfibrinogenemia appear to suffer an increased rate of pregnancy complications [54]. It is postulated that dysfibrinogens may increase the risk of thrombosis either by producing clots that are more resistant to fibrinolysis or by failing to sequester normal amounts of thrombin resulting in increased amounts of the free enzyme. Several mutations at or close to the thrombin cleavage sites have been reported in association with thrombosis (Marburg, Malmö, New York, and Naples), presumably via the latter mechanism [55]. The best described association with thrombosis is that of Fibrinogen Dusart Arg554Cys (also called Paris V and Chapel Hill III) as it has occurred several times [56]. The new Cys residue cross-links with albumin, resulting in formation of thin but plasmin-resistant fibrin fibers [57]. Similar phenomena have been reported in other dysfibrinogens with free thiol groups [58,59]. In one case, that of fibrinogen Oslo I [60], the dysfibrinogen was associated with thrombosis, shortened clotting times, and enhanced platelet activation.

Dysfibrinogens associated with bleeding

Some dysfibrinogens, particularly those with fibrinogen levels <1 g/L are associated with an increased frequency of bleeding. The mechanisms are diverse, e.g., Fibrinogen Bremen (Gly17Val) results in delayed polymerization and symptomatic bleeding as a result of impaired interaction of the "A" and "a" sites [61]. Many other examples can be found in the database. The symptoms are usually mild and may be associated with wound dehiscence. For those patients with a history of bleeding associated with a dysfibrinogen or hypofibrinogen, tranexamic acid or replacement therapy may be needed as for afibrinogenemia.

Dysfibrinogens associated with amyloidosis

Several mutations in the Aa chain gene have been found to cause hereditary renal amyloidosis (R554L, E526V, and 4904delG and 4897delT, which both lead to premature termination at codon 548). The fibrinogen behaves normally from a coagulation point of view and cannot be detected by this means. The role of fibrinogen was originally revealed by sequence analysis of the amyloid deposits [62]. The only curative approach appears to be liver transplantation [63].

Acquired dysfibrinogenemia

An acquired abnormality of fibrinogen typically arises in association with liver disease, especially hepatocellular carcinoma, owing to an increased number of sialic acid residues. The thrombin time is prolonged but the Clauss fibrinogen assay is normal or more frequently elevated. This is not associated with any bleeding tendency and can be ignored. A similar abnormality of fibrinogen is seen in neonates reflecting hepatic immaturity. Occasionally, laboratory results simulating the presence of a dysfibrinogenemia may be produced by a paraprotein or autoantibody interfering with fibrin polymerization. Although the fibrinogen may in fact be normal, this can nonetheless result in an hemorrhagic tendency.

References

1 Medved L, Weisel JW, Fibrinogen and Factor XSoSSCoISoTaH. Recommendations for nomenclature on fibrinogen and fibrin. *J Thromb Haemost* 2009; **7**: 355–9.
2 Doolittle RF. X-ray crystallographic studies on fibrinogen and fibrin. *J Thromb Haemost* 2003; **1**: 1559–65.
3 Tennent GA, Brennan SO, Stangou AJ, O'Grady J, Hawkins PN, Pepys MB. Human plasma fibrinogen is synthesized in the liver. *Blood* 2007; **109**: 1971–4.
4 Sahni A, Simpson-Haidaris PJ, Sahni SK, Vaday GG, Francis CW. Fibrinogen synthesized by cancer cells augments the proliferative effect of fibroblast growth factor-2 (FGF-2). *J Thromb Haemost* 2008; **6**: 176–83.
5 Tybjaerg-Hansen A, Agerholm-Larsen B, Humphries SE, Abildgaard S, Schnohr P, Nordestgaard BG. A common mutation (G-455→A) in the beta-fibrinogen promoter is an independent predictor of plasma fibrinogen, but not of ischemic heart disease. A study of 9127 individuals based on the Copenhagen City Heart Study. *J Clin Invest* 1997; **99**: 3034–9.

6 Laffan MA. Fibrinogen polymorphisms and disease. *Eur Heart J* 2001; **22**: 2224–6.

7 Humphries SE, Henry JA, Montgomery HE. Gene–environment interaction in the determination of levels of haemostatic variables involved in thrombosis and fibrinolysis. *Blood Coagul Fibrinolysis* 1999; **10**(Suppl. 1): S17–21.

8 Rox JM, Muller J, Potzsch B. Absence of fibrinogen alpha-, beta- and gamma-chain mRNA in human platelets. *Br J Haematol* 2005; **130**: 647–8.

9 Coller BS, Seligsohn U, West SM, Scudder LE, Norton KJ. Platelet fibrinogen and vitronectin in Glanzmann thrombasthenia: evidence consistent with specific roles for glycoprotein IIb/IIIA and alpha v beta 3 integrins in platelet protein trafficking. [comment]. *Blood* 1991; **78**: 2603–10.

10 Belloc F, Heilmann E, Combrie R, Boisseau MR, Nurden AT. Protein synthesis and storage in human platelets: a defective storage of fibrinogen in platelets in Glanzmann's thrombasthenia. *Biochimica et Biophysica Acta* 1987; **925**: 218–25.

11 Mullin JL, Gorkun OV, Binnie CG, Lord ST. Recombinant fibrinogen studies reveal that thrombin specificity dictates order of fibrinopeptide release. *J Biol Chem* 2000; **275**: 25239–46.

12 Gorkun OV, Veklich YI, Medved LV, Henschen AH, Weisel JW. Role of the alpha C regions of fibrin in clot formation. *Biochemistry* 1994; **33**: 6986–97.

13 Shainoff JR, Dardik BN. Fibrinopeptide B in fibrin assembly and metabolism: physiologic significance in delayed release of the peptide. *Ann NY Acad Sci* 1983; **408**: 254–68.

14 Mosesson MW. Fibrinogen and fibrin polymerization: appraisal of the binding events that accompany fibrin generation and fibrin clot assembly. *Blood Coagul Fibrinolysis* 1997; **8**: 257–67.

15 Lovely RS, Falls LA, Al-Mondhiry HA, *et al.* Association of gammaA/gamma′ fibrinogen levels and coronary artery disease. *Thromb Haemost* 2002; **88**: 26–31.

16 Spraggon G, Everse SJ, Doolittle RF. Crystal structures of fragment D from human fibrinogen and its crosslinked counterpart from fibrin.[erratum appears in *Nature* 1997; **390**: 315]. *Nature* 1997; **389**: 455–62.

17 Shainoff JR, Urbanic DA, DiBello PM. Immunoelectrophoretic characterizations of the cross-linking of fibrinogen and fibrin by factor XIIIa and tissue transglutaminase. Identification of a rapid mode of hybrid alpha-/gamma-chain cross-linking that is promoted by the gamma-chain cross-linking. *J Biol Chem* 1991; **266**: 6429–37.

18 Medved L, Nieuwenhuizen W. Molecular mechanisms of initiation of fibrinolysis by fibrin. *Thromb Haemost* 2003; **89**: 409–19.

19 Mosesson MW, Siebenlist KR, Hernandez I, Lee KN, Christiansen VJ, McKee PA. Evidence that alpha2-antiplasmin becomes covalently ligated to plasma fibrinogen in the circulation: a new role for plasma factor XIII in fibrinolysis regulation. *J Thromb Haemost* 2008; **6**: 1565–70.

20 Holmback K, Danton MJ, Suh TT, Daugherty CC, Degen JL. Impaired platelet aggregation and sustained bleeding in mice lacking the fibrinogen motif bound by integrin alpha IIb beta 3. *EMBO J* 1996; **15**: 5760–71.

21 Suehiro K, Gailit J, Plow EF. Fibrinogen is a ligand for integrin alpha5beta1 on endothelial cells. *J Biol Chem* 1997; **272**: 5360–6.

22 Yokoyama K, Erickson HP, Ikeda Y, Takada Y. Identification of amino acid sequences in fibrinogen gamma-chain and tenascin C C-terminal regions critical for binding to integrin alpha vbeta 3. *J Biol Chem* 2000; **275**: 16891–8.

23 Andersson RG, Saldeen K, Saldeen T. A fibrin(ogen) derived pentapeptide induces vasodilation, prostacyclin release and an increase in cyclic AMP. *Thromb Res* 1983; **30**: 213–18.

24 Gerdin B, Lindeberg G, Ragnarsson U, Saldeen T, Wallin R. Structural requirements for microvascular permeability-increasing ability of peptides. Studies on analogues of a fibrinogen pentapeptide fragment. *Biochimica et Biophysica Acta* 1983; **757**: 366–70.

25 Fukao H, Matsumoto H, Ueshima S, Okada K, Matsuo O. Effects of fibrin on the secretion of plasminogen activator inhibitor-1 from endothelial cells and on protein kinase C. *Life Sci* 1995; **57**: 1267–76.

26 Languino LR, Duperray A, Joganic KJ, Fornaro M, Thornton GB, Altieri DC. Regulation of leukocyte-endothelium interaction and leukocyte transendothelial migration by intercellular adhesion molecule 1-fibrinogen recognition. *Proc Natl Acad Sci USA* 1995; **92**: 1505–9.

27 Peng H, Sahni A, Fay P, *et al.* Identification of a binding site on human FGF-2 for fibrinogen. *Blood* 2004; **103**: 2114–20.

28 Whitton CM, Sands D, Hubbard AR, Gaffney PJ. A collaborative study to establish the 2nd International Standard for Fibrinogen, Plasma. *Thromb Haemost* 2000; **84**: 258–62.

29 Mackie IJ, Kitchen S, Machin SJ, Lowe GD, Haemostasis, Thrombosis Task Force of the British Committee for Standards in H. Guidelines on fibrinogen assays. *Br J Haematol* 2003; **121**: 396–404.

30 Dempfle C-E, Kalsch T, Elmas E, *et al.* Impact of fibrinogen concentration in severely ill patients on mechanical properties of whole blood clots. *Blood Coagul Fibrinolysis* 2008; **19**: 765–70.

31 de Maat MM, Lowe GDO, Haverkate F. Fibrinogen. In: Jespersen J, Bertina R, Haverkate F (eds.) *Laboratory Techniques in Thrombosis. A Manual*. Dordrecht, NL: Kluwer Academic Publishers, 1999: 79–88.

32 Kalina U, Stohr H-A, Bickhard H, *et al.* Rotational thromboelastography for monitoring of fibrinogen concentrate therapy in fibrinogen deficiency. *Blood Coagul Fibrinolysis* 2008; **19**: 777–83.

33 Mackie J, Lawrie AS, Kitchen S, *et al.* A performance evaluation of commercial fibrinogen reference preparations and assays for Clauss and PT-derived fibrinogen. *Thromb Haemost* 2002; **87**: 997–1005.

34 Lak M, Keihani M, Elahi F, Peyvandi F, Mannucci PM. Bleeding and thrombosis in 55 patients with inherited afibrinogenaemia. *Br J Haematol* 1999; **107**: 204–6.

35 Suh TT, Holmback K, Jensen NJ, *et al.* Resolution of spontaneous bleeding events but failure of pregnancy in fibrinogen-deficient mice. *Genes Dev* 1995; **9**: 2020–33.

36 Xiao Q, Danton MJ, Witte DP, Kowala MC, Valentine MT, Degen JL. Fibrinogen deficiency is compatible with the development of atherosclerosis in mice. *J Clin Invest* 1998; **101**: 1184–94.

37 Chafa O, Chellali T, Sternberg C, Reghis A, Hamladji RM, Fischer AM. Severe hypofibrinogenemia associated with bilateral ischemic necrosis of toes and fingers. *Blood Coagul Fibrinolysis* 1995; **6**: 549–52.

38 Neerman-Arbez M. Fibrinogen gene mutations accounting for congenital afibrinogenemia. *Annals of the New York Academy of Sciences* 2001; **936**: 496–508.

39 Neerman-Arbez M. The molecular basis of inherited afibrinogenaemia. *Thromb Haemost* 2001; **86**: 154–63.

40 Asselta R, Duga S, Tenchini ML. The molecular basis of quantitative fibrinogen disorders. *J Thromb Haemost* 2006; **4**: 2115–29.

41 Vu D, Neerman-Arbez M. Molecular mechanisms accounting for fibrinogen deficiency: from large deletions to intracellular retention of misfolded proteins. *J Thromb Haemost* 2007; **5**(Suppl. 1): 125–31.
42 Duga S, Asselta R, Santagostino E, *et al.* Missense mutations in the human beta fibrinogen gene cause congenital afibrinogenemia by impairing fibrinogen secretion. *Blood* 2000; **95**: 1336–41.
43 Keeling D, Tait C, Makris M. Guideline on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders. A United Kingdom Haemophilia Center Doctors' Organisation (UKHCDO) guideline approved by the British Committee for Standards in Haematology. *Haemophilia* 2008; **14**: 671–84.
44 Doyle S, O'Brien P, Murphy K, Fleming C, O'Donnell J. Coagulation factor content of solvent/detergent plasma compared with fresh-frozen plasma. *Blood Coagul Fibrinolysis* 2003; **14**: 283–7.
45 Negrier C, Rothschild C, Goudemand J, *et al.* Pharmacokinetics and pharmacodynamics of a new highly secured fibrinogen concentrate. *J Thromb Haemost* 2008; **6**: 1494–9.
46 Kreuz W, Meili E, Peter-Salonen K, *et al.* Pharmacokinetic properties of a pasteurised fibrinogen concentrate. *Transfus Apher Sci* 2005; **32**: 239–46.
47 Parameswaran R, Dickinson JP, de Lord S, Keeling DM, Colvin BT. Spontaneous intracranial bleeding in two patients with congenital afibrinogenaemia and the role of replacement therapy. *Haemophilia* 2000; **6**: 705–8.
48 Neerman-Arbez M, Honsberger A, Antonarakis SE, Morris MA. Deletion of the fibrinogen [correction of fibrogen] alpha-chain gene (FGA) causes congenital afibrogenemia.[erratum appears in *J Clin Invest* 1999; **103**: 759]. *J Clin Invest* 1999; **103**: 215–18.
49 Peyvandi F, Haertel S, Knaub S, Mannucci PM. Incidence of bleeding symptoms in 100 patients with inherited afibrinogenemia or hypofibrinogenemia. *J Thromb Haemost* 2006; **4**: 1634–7.
50 Ra'anani P, Levi Y, Varon D, Gitel S, Martinowitz U. Congenital afibrinogenemia with bleeding, bone cysts and antibodies to fibrinogen. *Harefuah* 1991; **121**: 291–3.
51 Kobayashi T, Kanayama N, Tokunaga N, Asahina T, Terao T. Prenatal and peripartum management of congenital afibrinogenaemia. *Br J Haematol* 2000; **109**: 364–6.
52 de Moerloose P, Neerman-Arbez M. Treatment of congenital fibrinogen disorders. *Expert Opin Biol Ther* 2008; **8**: 979–92.
53 Matsuda M, Sugo T, Yoshida N, *et al.* Structure and function of fibrinogen: insights from dysfibrinogens. *Thromb Haemost* 1999; **82**: 283–90.
54 Haverkate F, Samama M. Familial dysfibrinogenemia and thrombophilia. Report on a study of the SSC Subcommittee on Fibrinogen. *Thromb Haemost* 1995; **73**: 151–61.
55 Koopman J, Haverkate F, Lord ST, Grimbergen J, Mannucci PM. Molecular basis of fibrinogen Naples associated with defective thrombin binding and thrombophilia. Homozygous substitution of B beta 68 Ala–Thr. *J Clin Invest* 1992; **90**: 238–44.
56 Wada Y, Lord ST. A correlation between thrombotic disease and a specific fibrinogen abnormality (A alpha 554 Arg→Cys) in two unrelated kindred, Dusart and Chapel Hill III. *Blood* 1994; **84**: 3709–14.
57 Koopman J, Haverkate F, Grimbergen J, *et al.* Molecular basis for fibrinogen Dusart (A alpha 554 Arg→Cys) and its association with abnormal fibrin polymerization and thrombophilia. *J Clin Invest* 1993; **91**: 1637–43.
58 Vakalopoulou S, Mille-Baker B, Mumford A, Manning R, Laffan M. Fibrinogen Bbeta14 Arg→Cys: further evidence for a role in thrombosis. *Blood Coagul Fibrinolysis* 1999; **10**: 403–8.
59 Koopman J, Haverkate F, Grimbergen J, *et al.* Abnormal fibrinogens IJmuiden (B beta Arg14—Cys) and Nijmegen (B beta Arg44—Cys) form disulfide-linked fibrinogen-albumin complexes. *Proc Natl Acad Sci USA* 1992; **89**: 3478–82.
60 Egeberg O. Inherited fibrinogen abnormality causing thrombophilia. *Thrombosis et Diathesis Haemorrhagica* 1967; **17**: 176–87.
61 Wada Y, Niwa K, Maekawa H, *et al.* A new type of congenital dysfibrinogen, fibrinogen Bremen, with an A alpha Gly-17 to Val substitution associated with hemorrhagic diathesis and delayed wound healing. *Thromb Haemost* 1993; **70**: 397–403.
62 Hamidi Asl L, Liepnieks JJ, Uemichi T, *et al.* Renal amyloidosis with a frame shift mutation in fibrinogen aalpha-chain gene producing a novel amyloid protein. *Blood* 1997; **90**: 4799–805.
63 Gillmore JD, Booth DR, Rela M, *et al.* Curative hepatorenal transplantation in systemic amyloidosis caused by the Glu526Val fibrinogen alpha-chain variant in an English family.[comment]. *QJM* 2000; **93**: 269–75.

56 Miscellaneous bleeding disorders

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Introduction

The primary function of the coagulation system is to maintain hemostasis, prevent uncontrolled clot propagation, and assist in restoration of normal vascular architecture. Hemostasis involves a series of complex physiologic processes related to prevention of blood loss and the maintenance of both vascular integrity and blood fluidity. Upon vascular injury, coagulation is activated by expression of tissue factor locally and amplified through feedback mechanisms including contact factor activation resulting in a thrombus formation (Figure 56.1). There exist finely orchestrated interactions between the vascular endothelium, platelets/von Willebrand factor, and procoagulant proteins that are crucial to the formation of a thrombus at the site of vessel wall injury. Coagulation is controlled through naturally occurring anticoagulants and the fibrinolytic system; natural anticoagulants control thrombin generation by direct inhibition or inactivation of procoagulants while the fibrinolytic system restores normal architecture through gradual clot dissolution. Thus, qualitative and quantitative defects of platelets, the vessel wall, and coagulation and fibrinolytic systems can result in a bleeding diathesis. Bleeding disorders owing to congenital deficiencies of coagulation proteins including factors VIII, IX, XI, V, X, prothrombin, fibrinogen, and von Willebrand factor have been discussed in earlier chapters. This chapter will briefly discuss miscellaneous inherited disorders of the fibrinolytic system, platelets, and vessel wall that present with bleeding symptoms or a hemorrhagic diathesis. In addition, the deficiency states of contact factors within the kallikrein–kinin system have also been included. It is important to clarify that contact factor pathway deficiencies do not result in clinical bleeding symptoms but rather with isolated prolongation of screening coagulation tests and paradoxically with hypercoagulable state. These disorders deserve consideration during interpretation of abnormal coagulation tests. Deficiency of naturally occurring anticoagulant proteins predisposes to thrombosis and is beyond the scope of this chapter.

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Inherited deficiencies of fibrinolytic system

Overview of fibrinolytic system

Fibrinolysis is a complex process regulating both hemostasis and thrombosis [1]. The fibrinolytic pathway contains an inactive proenzyme, plasminogen, which requires conversion into an active enzyme, plasmin, to degrade fibrin into soluble degradation products (Figure 56.1) [2]. Plasminogen is converted into plasmin by two immunologically distinct physiologic plasminogen activators: tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). Inhibition of fibrinolysis occurs at the levels of plasminogen activators, via specific plasminogen activator inhibitors (PAI-1 and PAI-2) and at the level of plasmin, mainly through α_2-antiplasmin (Figure 56.1). Tissue-type plasminogen activator-mediated plasminogen activation is primary to the dissolution of fibrin in the circulation while u-PA binds to a specific cellular receptor (u-PAR) resulting in enhanced activation of cell-bound plasminogen. In normal plasma t-PA activity is extremely low and the majority of t-PA is in complex with PAI-l. Regulation and control of the fibrinolytic system is mediated through synthetic control and release of plasminogen activators and plasminogen activator inhibitors, primarily from endothelial cells. Hyperfibrinolysis owing to excessive synthesis or insufficient downregulation of plasmin is associated with a bleeding diathesis. To date, deficiencies of two fibrinolytic proteins, PAI-1 and α_2-antiplasmin (α_2-AP), have been known to be associated with a bleeding diathesis.

Congenital PAI-1 deficiency

Role of PAI-1 in fibrinolysis

Plasminogen activator inhibitor 1 (PAI-1) is a single-chain glycoprotein with a molecular weight of 52 kDa consisting of 379 amino acids preceded by a signal peptide of 23 amino acids. PAI-1 controls the proteolytic action of plasmin through inhibition of plasminogen activators, t-PA and u-PA. The PAI-1 gene has been mapped at 7q21.3–q22 locus (OMIM

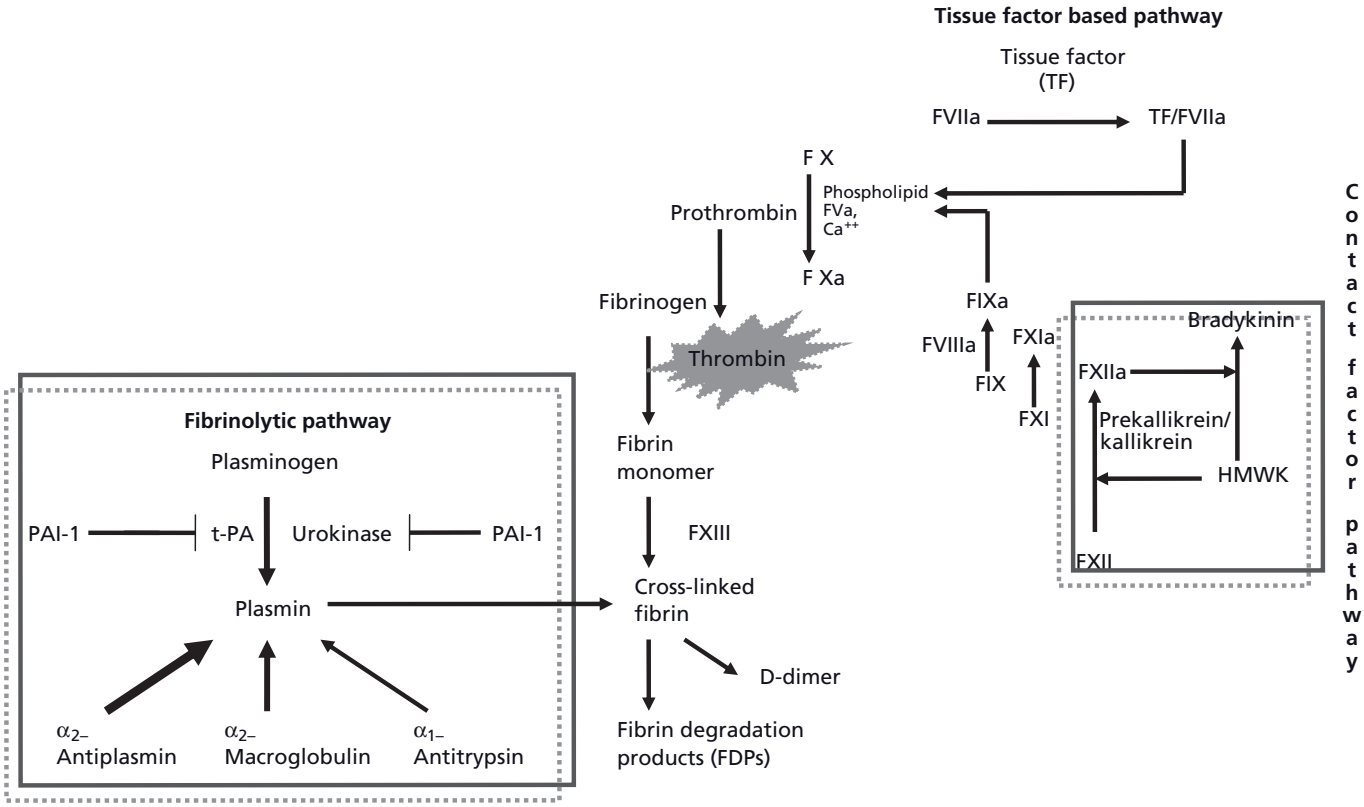

Figure 56.1 Coagulation pathways depicting interaction between fibrinolytic pathway and contact factor pathway. F, factor; PAI-1, plasminogen activator inhibitor 1; t-PA, tissue plasminogen activator.

#173360) and consists of nine exons and eight introns distributed over 12.2 kb of DNA [3]. In healthy individuals, there exist highly variable plasma antigenic levels of PAI-1 ranging from 6 to 85 ng/mL (geometric mean, 24 ng/mL) [4]. PAI-1 exhibits a circadian variation with a peak plasma concentration in the morning and troughs in the late afternoon and evening [5]. It is a relatively unstable molecule with both a latent and active conformational form. PAI-1 spontaneously inactivates under physiologic conditions with an *in vitro* half-life of 1–2 h. Binding of PAI-I to vitronectin, a cellular adhesion molecule from the extracellular matrix, stabilizes the active conformation, prolonging the half-life [6]. In humans, PAI-1 is present in human plasma, platelet α-granules, the placenta, and extracellular matrix. Presently, the origin of plasma PAI-1 has not been completely elucidated, but most likely originates from endothelial cells and/or hepatocytes. Synthesis and secretion of PAI-1 can be modulated by various chemical mediators including hormones, growth factors, endotoxin, cytokines, and phorbol esters. PAI-1 belongs to the family of serine protease inhibitors (serpin) and inhibits both single-chain and two-chain t-PA, and only single-chain u-PA through formation of a 1:1 stoichiometric complex through its reactive peptide bond, Arg346–Met347 [7]. The majority of t-PA in plasma is in complex with PAI-1; the level of active PAI-1 in the blood is an important regulator of the concentration, half-life, and circadian variation of active t-PA.

Clinical presentation of congenital PAI-1 deficiency

PAI-1 deficiency may result from either a quantitative (decreased or absent protein) [8,9] or qualitative defect (detectable protein with reduced or absent functional activity) [10,11]. In quantitative PAI-1 deficiency, affected patients carry one (heterozygote) or two (homozygote) alleles with a mutation, resulting in partial or total antigenic deficiency of PAI-1. It is important to clarify that heterozygous PAI-1 deficiency is not associated with bleeding symptoms even after trauma or surgery. Both partial and total PAI-1 deficiencies are extremely rare disorders; hence the prevalence of this condition in the general population is not established. PAI-1 deficiency is transmitted in an autosomal recessive inheritance pattern. Fay *et al.* [8] reported an Amish pedigree of 19 individuals with quantitative deficiency of PAI-1 that carried a homozygous null mutation in PAI-1 gene. These patients had a two base pair (TA) insertion at 3′ end of exon 4 in the PAI-1 gene causing a frame-shift in exon 4 resulting in early truncation of the PAI-1 protein with a correspondingly absent PAI-1 antigen in both platelets and plasma. Interestingly, isolated

Table 56.1 Characteristics of miscellaneous bleeding disorders of coagulation system.

	Plasminogen activator inhibitor-1 (PAI-1)	α_2-Plasmin inhibitor (α_2-PI)	Prekallikrein (PK)	High-molecular-weight kininogen (HMWK)	Factor XII
Synonyms		α_2-Antiplasmin deficiency	Fletcher factor	Fitzgerald factor Williams factor Flaujeac factor	Hageman factor
Gene map locus	7q21.3–q22	17pter–p12	4q35	3q27	5q33–qter
Molecular weight (daltons)	52 000	70 000	85 000–88 000	120 000	80 000–90 000
Normal plasma concentration (μg/mL)	24 ng/mL	0.7 ± 0.06 mg/L	35–50 μg/mL	80 μg/mL	30 μg/mL
Plasma half-life	2–4 h	60 h	58 h		48 h
Mode of inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive, autosomal dominant
Biochemical features	Single-chain glycoprotein, serine protease inhibitor	Single-chain glycoprotein, serine protease inhibitor	γ-Globulin, single-chain serine protease inhibitor	α-Globulin, single-chain cofactor	Glycoprotein, heavy-chain and light-chain serine protease inhibitor
Reactive site		Arg 364–Met 365 peptide bond	HMWK binding: Apple domains 1(F56–G86) and 4 (K266–G295) FXII binding: A3, A4		Arg353–Val354
Site of synthesis	Liver, megakaryocytes	Liver	Liver	Liver, human umbilical vein endothelial cells	Liver
Clinical features of deficiency state	Bleeding in homozygotes; morphologic/ developmental abnormalities absent	Bleeding in homozygotes	No clinical abnormalities	No clinical abnormalities	No clinical abnormalities, rarely migratory thrombophlebitis
Screening tests	Euglobulin clot lysis assay, whole-blood clotting assays	Euglobulin clot lysis assay, whole-blood clotting assays	aPTT, mixing studies	aPTT, mixing studies	aPTT, mixing studies
Confirmatory laboratory assays	PAI-1 antigen (ELISA) and activity (chromogenic) assay; gene mutation analysis	α2-antiplasmin antigen and activity assay; gene mutation analysis	PK antigen (ELISA) assay	HMWK assay (ELISA)	FXII assay (chromogenic)
Prophylaxis and treatment	Antifibrinolytics FFP as required Supportive care	Antifibrinolytics FFP as required Supportive care	None required	None required	None required, anticoagulation for thrombophlebitis

deficiency of plasma PAI-1 with normal platelet PAI-1 has been reported, questioning the role of PAI-1 in the platelet compartment. There is some suggestion that platelet PAI-1 may be protective against premature clot lysis early in coagulation but may not be available later when delayed bleeding occurs [6]. Thus, it can be speculated that the precise local concentrations of PAI-1 and the dynamic time course are important in controlling hemorrhage.

Although the literature on PAI-1 deficiency is limited, a report by Fay *et al.* [8] provides the most comprehensive information available about the phenotype of PAI-1 deficiency. The observed spectrum of bleeding episodes ranged from intracranial and joint bleeding after injury or mild trauma, delayed surgical bleeding, severe menorrhagia, and frequent bruising (Table 56.1). The clinical manifestations of abnormal bleeding were restricted to trauma or surgery in homozygous affected individuals; no bleeding manifestations were observed in heterozygotes [8]. The clinical manifestations in homozygous individuals are consistent with increased fibrinolytic activity, resulting in early destruction and detachment of the normal fibrin clot prior to adequate wound healing. In general, bleeding is expected to be more pronounced after trauma/surgical procedures especially involving the oral and urogenital areas owing to the increased concentration of local fibrinolytic activity in saliva and urine respectively. In regards to qualitative deficiencies of PAI-1, to

date there appears to be one case report in an elderly male presenting with a delayed bleeding diathesis after transurethral prostatic resection [11]. Similar to quantitative PAI-1 deficiency, this patient had a lifelong history of delayed postoperative bleeding and bleeding after trauma [11]. His euglobulin clot lysis assay revealed a shortened clot lysis time (50 min with normal exhibiting no lysis at 2 h) which partially corrected (1 h 45 min) after addition of an antifibrinolytic medication (ε-aminocaproic acid). Extensive evaluation for a deficiency in the fibrinolytic pathway confirmed what appeared to be a functional deficiency of PAI-1 despite a normal PAI-1 antigen.

Diagnosis and management of congenital PAI-1 deficiency

Accurate diagnosis of PAI-1 deficiency is important as it is effectively managed with fibrinolytic inhibitors, thereby decreasing the need for blood product support and risk of uncontrolled hemorrhage [8,12]. Typically, hyperfibrinolytic bleeding is characterized by normal platelet function tests and tests of global coagulation. The euglobulin clot lysis assay and whole blood clotting assays [13], such as the thromboelastogram, are helpful in diagnosis of hyperfibrinolytic states but are insufficient to confirm PAI-1 deficiency whose diagnosis is based on the measurement of antigenic [enzyme-linked immunosorbent assay (ELISA)] and functional (chromogenic test) PAI-1 assays. The major limitation of the currently available activity assay is that it is accurate in detecting elevated levels of PAI-1 but not at the lowest ranges. Above all, because of the variability of the PAI-1 levels within the normal population the lower range of PAI-1 activity assay includes zero, making the diagnosis of deficiency state extremely challenging. Owing to these limitations of activity assays, unless the genetic alteration leads to complete absence of the protein as in the cases reported by Fay *et al.* [8], the diagnosis of PAI-1 deficiency remains difficult and at times is assumed rather than proven. Hence, genotypic analysis may be helpful when available.

Since bleeding episodes in patients with PAI-1 deficiency result from injury and surgery, taking appropriate precautions to prevent bleeding is the best strategy to prevent life-threatening hemorrhage. Supportive care is helpful to ameliorate bleeding (Table 56.2). In addition, bleeding episodes may be effectively treated with antifibrinolytic agents including oral tranexamic acid or ε-aminocaproic acid; treatment duration is dependent upon the severity of the bleeding episode (Table 56.3). Persistent excessive menorrhagia may be managed with hormonal suppression and long-term prophylactic antifibrinolytic therapy if necessary. Severe bleeding events, including intracranial hemorrhage with or without hematoma evacuation, may be managed with intravenous fibrinolytic inhibitors; use of fresh-frozen plasma (dose 10–15 mL/kg) may be utilized to raise PAI-1 activity for initiation of therapy prior to achievement of steady-state levels of antifibrinoltyics. The need for ongoing treatment with fresh-frozen plasma is dependent on the individual patient's circumstances and response to medication therapy. To date, there are no reports of inhibitory antibody development against exogenously administered PAI-1 in congenital PAI-1 deficiency. Owing to the significant limitations in diagnosis of PAI-1 deficiency, a clinical trial of antifibrinolytic agents should be considered when a high index of suspicion is present for PAI-1 deficiency and after all other known bleeding disorders have been excluded.

Currently, antenatal diagnosis of PAI-1 deficiency is not offered as, in general, PAI-1 deficiency has a mild bleeding phenotype compared with severe hemophilia.

Table 56.2 General principles of supportive care in patients with miscellaneous rare bleeding disorders.

General measures
Avoidance of trauma, contact sports, fastidious dental care, use of medic alert bracelet
Safety checks for infants and toddlers at home
Local measures
Local bleeding: local pressure, application of ice
Dental bleeding: local application of fibrin sealants
Epistaxis: local pressure, ice, nasal packing, application of gel foam, topical thrombin, cauterization, rhinoplasty, or arterial embolization
Gingival bleeding: regular dental care, topical antifibrinolytic agents
Specific therapies
Dental bleeding: topical fibrin glue/thrombin, use of DDAVP and antifibrinolytic drugs, dental splints to prevent blood loss accompanying the loss of deciduous teeth
Menorrhagia: hormone suppression therapy
Iron-deficiency anemia secondary to blood loss: iron supplementation
Surgery/child birth: involvement of hematology service, type and cross for blood transfusions, use of hemostatic agents such as DDAVP, antifibrinolytics, replacement of deficient coagulation proteins through fresh-frozen plasma (FFP), use of platelets in platelet function disorders
Surveillance for alloantibody formation and virus transmission if patient exposed to blood products
Life-threatening bleeding: consider using recombinant factor VIIa in addition to other supportive measures

α_2-Plasmin inhibitor (α_2-PI) deficiency

Role of α_2-plasmin inhibitor in fibrinolysis

α_2-Plasmin inhibitor (α_2-PI), also known as α_2-antiplasmin, is a single-chain glycoprotein consisting of 452 amino acids with a molecular weight of 70 kDa [14]. The primary function of α_2-PI is inhibition of the proteolytic action of plasmin by direct

Table 56.3 Commonly used hemostatic agents to control bleeding in patients with PAI-1 deficiency, α_1-antiplasmin deficiency, and inherited platelet function disorders.

	Aminocaproic acid	Tranexamic acid	Desmopressin (1-deamino-8-D-argine vasopressin; DDAVP)	rFVIIa
Mechanism of action	Inhibitor of plasminogen activation	Competitive inhibitor of plasminogen activation; noncompetitive plasmin inhibition at higher concentrations	Endogenous release of von Willebrand factor and other procoagulants from vascular endothelium (Weible–Palade bodies); increased stickiness or reactivity of platelets	When complexed with tissue factor, NovoSeven® can activate factor X to factor Xa, as well as factor IX to factor IXa. Factor Xa, in complex with other factors, then converts prothrombin to thrombin, which leads to the formation of a hemostatic plug by converting fibrinogen to fibrin and thereby inducing local hemostasis
Dosage forms	500-mg and 1-g tablets; 250-mg/mL syrup; 250-mg/mL injection in 20-cc and 100-cc vials	500-mg tablets (not available in the USA); 100-mg/mL injection	Intravenous: 4 µg/mL solution Intranasal: Stimate®, 4.5 mg/mL	Intravenous form available as single-use vials of 1.2 mg, 2.4 mg and 4.8 mg
Oral dosage	3 g every 6 h or 10 g orally followed by 5 g every 6 h	25 mg/kg three to four times/day; absorption not affected by food	Not available	No oral dosage form available
Pediatric dosage	200 mg/kg as loading dose orally followed by 100 mg/kg every 6 h. Alternatively, 50–100 mg/kg/dose	Limited data in children to date; dosing instructions for adults may be used	Intravenous: 0.3 µg/kg of a 4 µg/mL solution diluted to 30–50 mL in 0.9% saline, and infused over 30 min Intranasal: <50 kg: 150 µg; >50 kg: 300 µg Maximum three consecutive doses administered at intervals of 24 h	90–120 µg/kg bolus every 2–3 h
Monitoring parameters	Laboratory monitoring evaluation usually not performed	Laboratory monitoring evaluation usually not performed; higher concentrations may prolong thrombin time	Younger children or patients with other systemic disorders serum sodium monitoring (±serum and urine osmolality)	Laboratory monitoring evaluation not performed
Contraindications (absolute and relative)	DIC (disseminated intravascular coagulation); upper urinary tract bleeding; history of thrombosis or thrombophilia	Defective vision; subarachnoid hemorrhage; renal compromise: dose adjustment required; history of thrombosis or thrombophilia	Use is restricted in children <20 kg or age ≤3 years; cystic fibrosis; cardiac disease, renal disease and thrombosis; history of thrombosis or thrombophilia	Known hypersensitivity to any component of the drug; known hypersensitivity to mouse, hamster, or bovine proteins; history of thrombosis or thrombophilia
Adverse events	Urinary obstruction if used for upper gastrointestinal tract bleeding	Visual changes with prolonged treatment	Flushing, tachycardia; tachyphylaxis after three doses; hyponatremic seizure; use in patients with atherosclerosis may precipitate a cardiovascular event	Potential risk of thrombosis
Use in pregnancy[a]	Category C	Category B	Category B	Category C
Drug interactions	Concomitant use with oral contraceptives or estrogens may potentiate a hypercoagulable state	Concomitant use with oral contraceptives or estrogens may potentiate a hypercoagulable state	Concomitant use with oral contraceptives or estrogens may potentiate a hypercoagulable state	None known

[a]US Food and Drug Administration (FDA) safety category of a drug for use in pregnancy. Category B indicates that the drug is potentially safe in animal studies but there is a lack of adequate clinical data in humans, while category C indicates that animal studies have demonstrated adverse effects in fetus but there are no clinical data in humans.

inhibition of plasmin(ogen). The gene for α_2-PI is located at 17pter–p12 (OMIM# 262850) and contains 10 exons and nine introns spanning over 16 kb of DNA. In humans α_2-PI is synthesized primarily in the liver. High levels of α_2-PI m-RNA have been observed in proximal convoluted tubules, smooth muscles, placenta, and the central nervous system; the clinical significance of these findings are not yet well elucidated. The mature α_2-PI protein belongs to the serine protease inhibitor (Serpin) family and consists of NH_2-terminal Met (Met-form) [15]. During circulation, the mature protein loses its 12 amino-terminal residues and is converted to the NH_2-terminal Asn (Asn-form) [16]. The Asn-form is present in plasma as 60–70% of the total α_2-PI [15]. The reactive site, reacting with the active center of plasmin, consists of Arg376–Met377 (according to amino acid numbering of Met-form) [14]. α_2-PI has a strong affinity for plasmin(ogen) and noncovalently binds to the lysine-binding site (LBS) of plasminogen leading to inhibition of plasmin(ogen) [17]. LBSs are sites at which fibrin is also noncovalently bound. Hence, α_2-PI competitively inhibits the binding of plasminogen to fibrin [18]. In addition to inhibition of plasmin, α_2-PI inhibits plasmin(ogen) binding to fibrin. The inhibition of plasmin at the clot level involves covalent binding (cross-linking) of α_2-PI with the α-chains of fibrin that are catalyzed by factor XIII. The plasma concentration of α_2-PI is 0.7 ± 0.06 mg/L. It is also present in the platelet α-granules at low concentrations constituting only 0.05% of α_2-PI in the whole blood [19]. Its half-life is 2.6 days, whereas the half-life is 0.5 days for the plasmin–α_2–PI complex [20].

Clinical presentation of congenital α_2-plasmin inhibitor deficiency

Owing to the rarity of this disorder, the real prevalence is not clearly established. Based on the results of functional and immunologic assays, two types of biologic deficiencies have been reported: type I (quantitative) [21,22], defined by similar decrease in both antigen and activity, and type II (qualitative), in which there is discrepancy between the activity and antigen (lowered activity compared with normal antigen). The mode of inheritance is autosomal recessive.

Since the first case report of α_2-AP deficiency in 1979 by Koie *et al.* [21], a total of 15 [22] cases have been documented. Similar to PAI-1 deficiency, the bleeding manifestations are characterized by post-traumatic or -surgical bleeding and are attributed to premature hemostatic plug dissolution prior to vessel repair. Reported bleeding episodes are moderate to severe and often present in childhood. Hemorrhagic episodes in the reported cases include umbilical bleeding, prolonged bleeding from wounds, epistaxis, gingival bleeding, hematuria, subcutaneous and intramuscular hematomas, hemothorax, central nervous system bleeding, and hemarthroses. Interestingly, an unusual bleeding site, intramedullary hematoma in the diaphyses of the long bones, has been reported in patients with α_2-AP deficiency [23,24]. Radiography indicates homogenous hyperlucent lesions with well-defined margins without marginal sclerosis that may be difficult to distinguish from cystic fibrous dysplasia, Langerhans cell histiocytosis, or metastatic neuroblastoma. Accurate diagnosis of these intramedullary hematomas may be confirmed with magnetic resonance imaging revealing homogeneous hyperintense signal in the medulla and a hypointense signal surrounding the lesion. The fact that the spectrum of severity exhibited by homozygous or compound heterozygous affected individuals varies from severe to moderate may relate to the variability of genetic defects and their specific impact on enzymatic function and other, as yet unidentified, factors.

In general, homozygous (two abnormal alleles) affected individuals experience a significant bleeding tendency while clinical bleeding in heterozygous (one abnormal allele) individuals is a matter of controversy. The majority of heterozygous individuals are discovered through family studies of homozygous individuals and have not experienced a bleeding tendency. However, there are a few case reports that describe bleeding manifestations in heterozygous individuals [25,26]. Bleeding events in heterozygous individuals have been reported to occur after trauma, including surgery or dental extraction, and have occurred in children [25] and in adults [26]. There is a suggestion that the bleeding tendency may increase with age [27]. Intramedullary hematomas have not been reported in heterozygous individuals.

Diagnosis and management of congenital α_2-plasmin inhibitor deficiency

Diagnosis of α_2-PI requires a high index of suspicion as screening coagulation assays and platelet function tests are normal even in those affected with α_2-PI deficiency. The euglobulin clot lysis time and whole-blood clotting assays can be useful to indicate a hyperfibrinolytic state; functional and immunologic α_2-PI assays are required for diagnosis.

Supportive care and antifibrinolytic medications, including ε-aminocaproic acid and tranexamic acid, are the mainstay of treatment (Table 56.2). Fresh-frozen plasma (FFP) may be used as an alternative or as adjunct to antifibrinolytic therapy in specific situations when immediate increase of the clotting factor is required. Based on the observation of Yoshioka *et al.* [22], infusion of FFP (17.5 mL/kg) increases the plasma concentration of α_2-PI antigen and activity to 15.6% and 19%, respectively. The half-life of infused α_2-PI antigen and activity was 35.5 and 21 h, respectively [22]. It is important to emphasize that treatment with FFP may be ineffective in individuals with a reduced concentration of factor XIII (FXIII) activity as α_2-PI induced inhibition of fibrin-bound plasmin is catalyzed by FXIII [28]. Treatment of intramedullary hematomas may require curettage with local application of hemostatic agents, such as fibrin glue, along with systemic antifibrinolytic therapy.

Inherited deficiencies of contact factor or kallikrein–kinin system

Role of contact factors in the kallikrein–kinin system in hemostasis

The contact system, also known as kallikrein–kinin system, was first recognized as a plasma and tissue proteolytic system responsible for liberation of the vasoactive proinflammatory mediator bradykinin (BK) [29]. Since the initial discovery of BK, there has been an evolution in our understanding of the plasma "coagulation contact system" [30]. The components of the contact system include factor XII (Hageman factor), prekallikrein (PK; Fletcher factor), and high-molecular-weight kininogen (HK; Williams, Flaujeac, or Fitzgerald factor). These plasma proteins together were grouped as the contact system as they required contact with an artificial, negatively charged surface for zymogen activation. Physiologic activation of the contact system occurs when PK, in the presence of HK, bound to endothelial cells, is converted into active kallikrein. Subsequently, HK is activated, liberating BK. On endothelial cells, factor XII activation occurs secondary to PK activation, which in turn amplifies activation of PK and factor XII. Factor XII contributes to the rate and extent of PK activation on the surface of endothelial cells but is not the initiator of the process. The critically important substrates of activated factor XII include factor XI and the macromolecular complex of the first component of complement. Thus, factor XI activation proceeds after factor XII activation, which in turn activates the intrinsic coagulation pathway. It is important to note that activation of the intrinsic system occurs primarily through the tissue factor-dependent pathway and is not solely dependent on factor XII-mediated activation of factor XI; these concepts imply a limited role of contact system in physiologic hemostasis.

The primary function of the kinin–kininogen system is to synthesize BK, which in turn contributes to the signs of inflammation including erythema, capillary leak, and hypotension. Bradykinin potently stimulates t-PA liberation and nitric oxide formation. In addition, formed plasma kallikrein promotes single-chain urokinase activation and subsequent plasminogen activation, thus enhancing the fibrinolysis. Lastly, kininogens and their breakdown products are antithrombotic, which contribute to the constitutive anticoagulant nature of the intravascular compartment.

Clinical presentation of contact factor deficiencies

Similarities between homozygous deficiencies of contact system proteins include their rarity, autosomal recessive inheritance pattern, and significant prolongation of the activated partial thromboplastin time (aPTT) without clinical hemorrhagic events [31]. Paradoxically, contact system deficiencies have been reported to result in thrombosis because of their intimate relationship to activation of the fibrinolytic system. Migratory thrombophlebitis has been described in patients with severe factor XII deficiency [32]. Table 56.1 describes the biochemical, physiologic properties, and clinical presentation of deficiencies of HK, BK, kalikrein, and factor XII.

Diagnosis and management of contact factor deficiencies

The diagnosis of a deficiency of a contact factor is suspected based upon prolongation of the aPTT (often >80 s) in the absence of clinical bleeding and/or presence of hypercoagulability. Mixing studies with normal pooled plasma corrects the aPTT prolongation, implying an underlying factor deficiency. Specific factor assays that measure the antigenic level through an immunologic (ELISA) are available for HK and BK. Factor XII activity may be measured by a chromogenic assay. As these disorders do not result in clinical bleeding diatheses, no active medical treatment is required beyond patient and family counseling concerning the underlying deficiency. Patients presenting with thromboembolic complications or thrombophlebitis may require treatment with anticoagulation.

Inherited platelet function disorders

Role of platelets in hemostasis

Platelets are acellular complex organelles derived from the cytoplasm of megakaryocytes. The concentration of platelets in blood is approximately 150 000–450 000 cells/mL. In the normal physiologic state, platelets circulate without adhering to undisturbed vascular endothelium. Upon disruption of vascular endothelial integrity or alteration in blood flow shear stress, platelets are "activated." Platelet activation involves an orchestrated interaction between von Willebrand factor, subendothelial proteins including fibronectin, laminin, and platelet membrane receptors followed by intracellular signal transduction and release of platelet-dense and α-granular contents through reorganization of canalicular system; these complex interactions result in the primary hemostatic plug and provision of the optimal phospholipid surface for secondary hemostasis to proceed. Thus, inherited abnormalities of platelet membrane receptors, signal transduction pathways and deficiencies of α and dense granules result in platelet dysfunction and bleeding disorders (Figure 56.2 and Table 56.4). The prevalence of these disorders is not well established.

Clinical symptoms of inherited platelet dysfunction

Inherited platelet function disorders are a heterogeneous group of disorders with variable bleeding symptoms ranging from almost asymptomatic to severe bleeding manifestations [33]. In general, the bleeding phenotype is mild to moderate in severity and may present in early childhood. Commonly

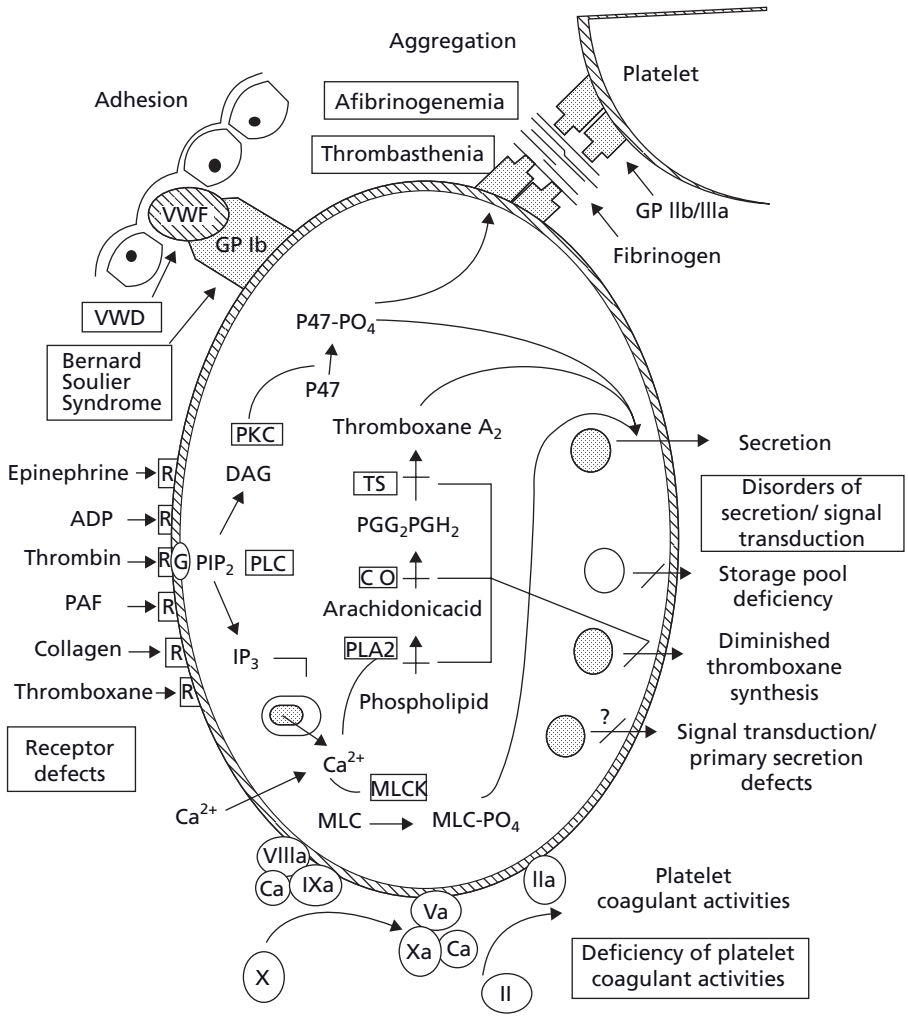

Figure 56.2 Schematic representation of inherited platelet function disorders. CO, cyclooxygenase; DAG, diacylglycerol; IP3, inositoltriphosphate; MLC, myosin light chain; MLCK, myosin light-chain kinase; PIP2, phosphatidylinositol bisphosphate; PKC, protein kinase C; PLC, phospholipase C; PLA2, phospholipase A2; VWF, von Willebrand factor; VWD, von Willebrand disorder. Reproduced with permission from Lippincott Williains & Wilkins.

encountered clinical symptoms include (i) unexplained or extensive bruising with or without soft-tissue hematomas; (ii) epistaxis, particularly episodes lasting more than 30 min, resulting in anemia, or requiring intervention or hospitalization; (iii) menorrhagia, particularly if present since menarche; (iv) gingival bleeding; (v) bleeding during or after childbirth; and (vi) bleeding following invasive procedures, e.g., dental extraction, tonsillectomy, adenoidectomy. Rarely, gastrointestinal bleeding, visceral hematoma, hemarthrosis, and intracerebral hemorrhage have been reported in conjunction with inherited platelet disorders such as Glanzmann thrombasthenia, although these bleeding symptoms are more commonly observed in hereditary or acquired coagulation factor disorders.

In a few cases, patients may present with other physical stigmata along with bleeding symptoms, e.g., presence of cataracts or hearing deficits as seen in *MYH-9* (nonmuscle myosine heavy chain 9) disorders, occulocutaneous albinism in patients with Hermnasky–Pudlak syndrome, and macrocephaly in children with Sebastian syndrome. A positive family history of bleeding symptoms further helps to strengthen the diagnosis of the inherited nature of the disorder. It is important to clarify that the absence of a family history of bleeding does not necessarily rule out the inherited nature as some disorders exhibit an autosomal recessive inheritance pattern. Table 56.4 provides a brief overview of inherited platelet function disorders.

Diagnosis of inherited platelet function disorders

A detailed clinical history, family history, and physical examination are crucial to the diagnosis of inherited platelet function disorders [33,34]. There is no single laboratory test that reliably predicts all platelet function abnormalities. A complete blood count with review of the peripheral smear is the initial step to evaluate a platelet function disorder. This basic test provides important information, including the platelet count and morphology of the cell lines critical for the diagnosis of various inherited platelet disorders. For example, large platelets are characteristic of *MYH-9* disorders while microthrombocytopenia is characteristic of Wiscott–Aldrich syndrome. In gray platelet syndrome, the dense granules are

Table 56.4 Inherited platelet function abnormalities.

Disorder	Platelet count ('000/ mm^3)	Inheritance	Structural defect	Platelet characteristics	Defect in platelet function	Associations	Treatment options		
							Platelet transfusion	DDAVP	r-FVIIa
Disorders of adhesion and aggregation owing to defects in receptors and defects in signal transduction									
Bernard–Soulier syndrome	20–100	AR	GPIb-IX GPIbα GPIbβ GPIX	Giant platelets	Abnormal adhesion	DiGeorge Velocardio facial syndrome	Y	Y	Y
Glanzmann thrombasthenia	Normal	AR	GPIIb/IIIa	None	Absent aggregation with physiologic agonists, defective clot retraction	↑ bone thickening and ↓ fertility	Y	N	Y
Platelet-type VWD	Normal or ↓	AD	GPIbα	Platelet size heterogeneity	Abnormal adhesion: ↑ sensitivity to ristocetin	Absence of HMWM	Y	N	?
$\alpha_2\beta_1$ collagen receptor	Normal	?	? α_2	Normal	Abnormal adhesion: ↓ response to collagen	Modifications in receptor density according to haplotype	Y	Y	?
GPVI collagen receptor	Normal	?	GPVI absence can be secondary to proteolytic cleavage	Normal	Abnormal adhesion: ↓ response to collagen	Linked to FcR-γ Receptor density depends upon haplotype	Y	Y	?
$P2Y_{12}$ ADP receptor	Normal	AR	$P2Y_{12}$ receptor	Normal	Abnormal aggregation to ADP	Not known	Y	Y	?
$TP_{\alpha,}$ thromboxane (TX) A_2 receptor	Normal	AR	TPα	Normal	Absence of response to TXA2 analogues, ↓ response to collagen	Not known	Y	Y	?
Intracellular signaling	Normal or ↓	?	Phospholipase C-β_2 $G_{\alpha q}$ protein among others	Normal	Variable aggregation and secretion defects on multiple agonists	Not reported	Y	Y	?
Cyclooxygenase deficiency	Normal or ↓	?AR	Cyclo-oxygenase enzyme	Not known	No aggregation with arachidonic acid, ↓ response to collagen and ADP	Not known	Y	Y	?
Scott syndrome	Normal	AR	ATP-binding cassette transporter A1	Normal	↓ procoagulant activity and microparticle release	Defects extend to other cell lines	Y	?	?
Wiscott–Aldrich syndrome	10–100	X-linked	WAS signaling defects	Small size, fewer granules	↓ aggregation and ↓ secretion	Eczema, immunodeficiency	Y	?	?

Disorders of secretion owing to abnormalities of storage granules									
Dense granule deficiency with albinism: Hermanasky–Pudlak (HP) Chediak–Higashi (CH) syndrome	Normal	AR	Proteins involved in vesicle formation and trafficking	↓ number of abnormal dense granules, giant granules (CH)	↓ aggregation and secretion with collagen	Oculocutaneous albinism, ceroid-lipofuscinosis (HP), infections (CH), pulmonary fibrosis (3rd–4th decade) and inflammatory bowel disease	Y	Y	Y
Gray platelet syndrome	30–100	AR or AD	Unknown, but prevents storage of proteins in α-granules	Empty α-granules	Abnormal but variable; can be decreased with thrombin, epinephrine and/or collagen	Myelofibrosis	Y	?	?
Quebec syndrome	~100	AD	↑ urokinase-type activator in α-granules, degraded proteins	Abnormal content of α-granules	Absent aggregation with epinephrine	None known	Y	?	?
Paris–Trousseau/ Jacobsen syndrome (deletion of 11q23–24)	30–150	AD	Defective megakaryopoiesis	Giant megakaryocyte granules	Abnormal aggregation and secretion with thrombin, epinephrine, ADP, and collagen	Psychomotor retardation, facial and cardiac abnormality	Y	Y	?
Dense granule deficiency without albinism	Normal	AD/X-R	Inability to package δ- granule contents	Quantitative deficiency of δ-granules, ↓ serotonin content	Absent second wave of aggregation with ADP, epinephrine ATP : ADP ratio >3	Wiscott–Aldrich syndrome, TAR, Ehler–Danlos syndrome	Y	Y	Y
MYH-9 disorders									
May–Hegglin	30–100	AD	MYH-9 Nonmuscle myosin heavy-chain IIA	Large size	No consistent defect	Neutrophil inclusions	Y	?	?
Fechtner syndrome	30–100	AD	MYH-9	Large size	No consistent defect	Hereditary nephritis, hearing loss	Y	?	?
Epstein syndrome	5–100	AD	MYH-9	Large size	Impaired response to collagen	Hereditary nephritis, hearing loss	Y	?	?
Montreal platelet syndrome	5–40	AD	Unknown	Large size	Spontaneous agglutination, ↓ response to thrombin	None known	Y	?	?

DDAVP, desmopressin (1-deamino-8-D-argine vasopressin); r-FVIIa, recombinant factor VIIa; AD, autosomal dominant; AR, autosomal recessive; ADP, adenosine diphosphate; ATP, adenosine triphosphate; HMWM, high-molecular-weight multimers; Y, yes; N, no; FcR-γ, Fc receptor-γ; TXA2, thromboxane A2; GP, glycoprotein; TAR, thrombocytopenia and absent radii; ?, indicates no documented efficacy; MYH-9, nonmuscle myosine heavy chain 9; WAS, Wiscott–Aldrich syndrome. Reprinted with permission from Sharathkumar AA, Shapiro AD, from World Federation of Hemophilia resource website.

absent and platelets look uniformly pale or "gray" on the peripheral smear.

The bleeding time (BT) and platelet function analysis with the PFA-100 system are screening tests to assess platelet function. The BT is the only *in vivo* test for platelet function but is operator dependent, affected by a subject's age and skin laxity, and is inconsistently reproducible. The PFA-100 (Dade-Behring, Marburg, Germany) is an *in vitro* BT test and is operator friendly. Both the BT and the PFA-100 closure time are prolonged in patients with low hematocrits and normal platelet function. Despite the limitations of the BT and PFA-100 closure time, they can be useful to narrow diagnostic considerations among patients with a history of bleeding.

Specific platelet function tests performed through platelet aggregation studies assess platelet aggregation to a panel of agonists, most commonly adenosine diphosphate, epinephrine, thrombin, collagen, arachidonic acid, and ristocetin, with platelet-rich plasma or through whole-blood aggregometry. The pattern obtained usually allows the investigator to diagnose and generally classify the defect. For example, von Willebrand disease (type IIb) and Bernard–Soulier syndrome may be associated with abnormalities in aggregation to ristocetin while Glanzmann thrombasthenia is associated with a flat aggregation profile to all agonists excluding ristocetin.

Platelet secretion is predominantly measured via two assays. Lumiaggregometry simultaneously measures aggregation and luciferase luminescence reflecting release or secretion of adenosine triphosphate (ATP) by the dense granules upon stimulation by agonists. Secretion can also be measured by allowing platelets to take up ^{14}C-labeled serotonin with subsequent release measured in response to agonists. These tests are technologically challenging and should be performed in specialized reference laboratories. Release and storage pool defects may show abnormalities via these secretion assays.

Flow cytometry, a technique that measures cellular protein expression through monocloncal antibodies, is available to measure platelet surface glycoproteins (GPs) and is used in clinical practice to diagnose Bernard–Soulier syndrome and Glanzmann thrombasthenia, which are associated with decreased or absent expression of GPIb/IXa/Va and GPIIb/IIIa, respectively. Electron microscopy is helpful to evaluate platelet granules including α or dense granular defects. Molecular analysis of platelet GPs and more specialized tests such as receptor expression analysis, protein phosphorylation, and formation of second messengers are available through dedicated research laboratories.

Management of inherited platelet function disorders

Predictors for bleeding risk in patients with inherited platelet disorders are lacking. Most bleeding experienced is an accentuation of normal anatomic and physiologic bleeding. Care should therefore be focused on prevention whenever possible. Important aspects of supportive care include avoidance of medications that may exacerbate platelet dysfunction such as aspirin or ibuprofen, maintaining excellent dental hygiene to prevent gingival bleeding, use of nasal packing/topical thrombin to control epistaxis, hormonal therapy for menorrhagia, and topical fibrin glue for dental extraction, to name a few. Caution regarding use of herbal medications should be employed as many may also interfere with hemostasis. Specific treatment modalities include use of hemostatic agents, including the antifibrinolytic agents ε-aminocaproic acid and tranexamic acid, with or without desmopressin (DDAVP), when a specific disorder is known to be responsive (Table 56.3) [35]. Surgical procedures involving areas with a high fibrinolytic activity, including the oropharynx (tonsillectomy and adenoidectomy, dental extractions) and urogenital area (e.g. prostatectomy), require careful preoperative planning including consideration for the need for repetitive doses of DDAVP and prolonged antifibrinolytic treatment to prevent catastrophic delayed bleeding. Occasionally, platelet transfusions may be required for hemostatic control. Patients with platelet disorders may be subject to repeated episodes of transfusion, placing them at risk for development of alloantibodies either to human leukocyte antigens (HLAs) or missing GPs as in Bernard–Soulier syndrome (GP Ib/IX/V) and Glanzmann thrombasthenia (GP IIb/IIIa). Formation of alloantibodies can result in platelet refractoriness and failure to obtain hemostasis. Hence, it is advised that platelet transfusion should be utilized judiciously in these patients; HLA-matched platelets should be employed unless the delay in obtaining these would compromise clinical outcome. Recently, there are reports of successful use of recombinant activated factor VII (rFVIIa; NovoSeven®, Novo Nordisk, Bagsvaerd, Denmark) in patients with Glanzmann thrombasthenia who developed antibodies against GP IIb/IIIa. Other treatment modalities including splenectomy have not been proven to reliably improve platelet counts in the majority of inherited platelet abnormalities except in Wiscott–Aldrich syndrome. Short courses of steroids can be beneficial to reduce mucocutaneous symptoms through improved vascular stability.

Bleeding disorders related to inherited vascular abnormalities

Role of blood vessels in hemostasis

Blood circulates in an intact vasculature which consists of arterial, venous, and capillary compartments. The vascular endothelium has a number of properties that directly or indirectly affect hemostatic balance. These include the expression of procoagulants and anticoagulants, adhesion molecules, vasoconstrictor and vasodilator substances, and cell survival signals. The quiescent endothelial cell lining of the vasculature promotes and maintains the blood fluidity by inhibiting coagulation and platelet adhesion and through stimulating

fibrinolysis. Upon vascular injury, the local and or systemic vasculature rapidly responds by "vasoconstriction," an important component of primary hemostasis that limits blood loss. Vasoconstriction assists in sealing the injured site yet maintaining blood flow to vital structures. Injury to the vascular wall is a powerful stimulus initiating coagulation, a pivotal process to control hemorrhage and repair the injured vessel wall. Structural and functional integrity of vasculature, therefore, is vital to maintain physiologic hemostasis. Inherited abnormalities of collagen, the major constituent of the extracellular vasculature matrix, represented by Ehler–Danlos syndrome vascular type (type IV) and disorders of angiogenesis such as Osler–Weber–Rendu syndrome (hereditary hemorrhagic telangiectasia) result in increased vascular fragility with a predisposition to overt and/or occult bleeding. These disorders require a high index of suspicion to avoid unnecessary laboratory evaluations and to identify appropriate disease specific management.

Ehlers–Danlos syndrome vascular type (type IV)

Ehlers–Danlos syndrome (EDS), also known as "cutis hyperelastica," "elastic skin," and "India rubber skin," is a group of rare genetic disorders caused by a defect in collagen synthesis [36]. These disorders are characterized by skin hyperelasticity, fragility of wound and blood vessels, delayed wound healing and joint hypermobility. There are a number of forms of EDS [37] with bleeding manifestations such as extensive ecchymoses more marked in type IV (EDS IV, OMIM #130050) because of pronounced dermal thinning.

Molecular mechanisms

EDS IV is caused by mutations in the type III procollagen gene *COL3A1* (OMIM# 120180) [38]. The *COL3A1* gene is located in chromosome band 2q32.2 [39]. This form may have autosomal dominant inheritance [40,41]. Varied molecular mechanisms have been observed and, of the mutations described to date, most have been unique to each family and thus considered "private," without correlation between genotype and phenotype. The estimated prevalence of EDS ranges between 1 in 10 000 to 1 in 25 000, without an ethnic predilection. EDS IV accounts for 5–10% of all of ED [42].

Clinical features

Type III collagen is abundant in the intestine and arterial wall, thus accounting for the most common initial clinical presentation for patients with EDS IV. The vascular complications may affect all anatomic sites, with a tendency toward large and medium diameter arteries. Dissections of the vertebral arteries and the carotids in extra- and intracranial segments (carotid–cavernous fistulae) are typical. Intestinal complications include spontaneous intestinal perforation. Paradoxically, joint hypermobility is less prominent and may be confined largely to the fingers with only mildly hyperextensile skin. Other EDS IV clinical presentations include premature birth, thin nose and lips, thin translucent skin with prominent venous markings, and small linear trunkal telangiectases, keloid formation, and rectal bleeding owing to multiple colonic diverticula [43]. Pregnancy-related complications included rupture of bowel, aorta, vena cava or uterus, vaginal laceration, and postpartum uterine hemorrhage [44]. The newborn period can be complicated by prematurity and spontaneous subarachnoid hemorrhage [45].

The most comprehensive study of EDS IV included 419 patients (220 index patients and 199 of their affected relatives) with confirmed biochemical abnormalities in type III collagen revealed that the average age of presentation was 23 years [43]. Arterial ruptures accounted for the majority of deaths, whilst digestive perforations, occurring mainly in the sigmoid colon, are less often fatal. These complications were rare in childhood, but 25% of index patients had medical and surgical complications before the age of 20, and more than 80% experienced such complications by the age of 40 years. The age at death ranged from 6 to 73 years, with a median lifespan of 48 years.

Diagnosis

A detailed history, including family history and thorough clinical examination, is important to establish the diagnosis. The most common abnormality noted in patients with EDS is a prolonged bleeding time owing to characteristics of the skin in the context of a normal blood count and screening coagulation tests. Rarely, an aspirin-like platelet function defect has been reported in patients with EDS IV [46]. Biochemical analysis of type III collagen from skin fibroblast culture has proven to be the most reliable diagnostic test [36]. Molecular genetic testing to identify mutations in *COL3A1* is available to patients with a biochemically confirmed diagnosis of vascular EDS [47]. Genetic testing is not routinely recommended for diagnosis of EDS IV as 40% of patients with biochemical defects in type III collagen have no identifiable mutation. In addition, the mutation does not predict the clinical disease severity.

Management

There is no specific treatment or cure for EDS IV but measures can be taken to minimize blood loss and discomfort. Although most affected patients with EDS IV commonly survive the first and second major complications, premature death because of catastrophic vascular and/or intestinal complications are observed. Hence, family counseling about EDS and anticipatory guidance is crucial. All patients should be offered genetic counseling particularly prior to pregnancy as there is an increased risk of uterine rupture.

Multidisciplinary follow-up involving a team of surgeons, radiologists, obstetricians, and geneticists for vascular/visceral/pregnancy complications is crucial to allow early intervention to avoid bleeding complications.

The role of hemostatic agents has not been carefully evaluated but seems to be beneficial. Tranexamic acid postoperatively has been utilized to reduce bleeding [48]. There are reports of improvement in the bleeding time in patients treated with DDAVP [49]. Prophylactic measures are of special importance, including avoidance of trauma including contact sports or isometric exercises. Proper wound repair is required to prevent cosmetic disfigurement. The surgeon should be knowledgeable of the diagnosis; extra sutures may be added and the period prior to removal lengthened by a few days. Avoidance of drugs which may cause or exacerbate platelet dysfunction such as aspirin and ibuprofen is prudent.

Hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu syndrome)

Hereditary hemorrhagic telangiectasia (HHT; also known as Osler–Weber–Rendu syndrome) is a relatively common, under-recognized autosomal dominant disorder that results from multisystem vascular dysplasia characterized by telangiectases and arteriovenous malformations (AVMs) of skin, mucosa, and viscera [50–52]. The individual lesion in HHT is known as "telangiectasis" (pl. telangiectases) while the process of formation of telangiectasia or telangiectasis is referred as "telangiectasia" (OMIM# 187300). The telangiectasis, small arteriovenous shunts involving both dilated arterioles and venules, appears as a 1–2-mm red spot on the skin and blanches upon slight pressure. HHT affects all ethnic and racial groups and is seen over a wide geographic distribution with an overall frequency of 1 in 5000 to 1 in 10 000 persons [53].

Molecular pathogenesis

Mutations in transforming growth factor-β/bone morphogenesis protein (TGF-β/BMP) signaling pathway, a pathway associated with angiogenesis, is involved in molecular pathogenesis of HHT. The proteins encoded by different mutated loci (*ALK1*, *ENG*, *BMPRII*, and *MADH4*) in HHT patients share functional roles in TGF-β/BMP pathway [54–56]. Mutations in the *ENG* gene (OMIM# 131193) localized on the long arm of chromosome 9 (9q33–q34.1) are responsible for HHT1 [57,58], whereas HHT2 results from mutations of the *ALK1* gene (OMIM# 601284) localized on the long arm of chromosome 12 (12q11–q14) [59]. Two additional loci, 5q31.3–q32 (OMIM# 601101) [60] and 7p14 (OMIM# 610655) [61], have been suspected but the genes of interest have not been identified. Association of HHT has been suspected in patients with juvenile polyposis coli and primary pulmonary hypertension.

Clinical manifestations of hereditary hemorrhagic telangiectases

Abnormal vessel formation and subsequent bleeding from these lesions is the basis of most clinical manifestations of HHT. Although the number and location of lesions vary widely, even within the same family, most telangiectases are found in the oral, nasal, and gastrointestinal mucosa and fingertips, whereas AVMs occur most commonly in the lungs, liver, and CNS. In general, smaller telangiectatic lesions usually present with symptoms of recurrent bleeding, whereas symptoms of the larger, internal AVMs do not result from hemorrhage. Complications of AVMs most often occur as a result of shunting of blood, thrombosis, or embolus. Table 56.5 describes the clinical manifestations of HHT according to the organ of involvement and its management.

Epistaxis because of telangiectases in the nasal mucosa is the most common and often the earliest symptom of HHT. As many as 95% of affected individuals eventually experience recurrent epistaxis, with a mean age at onset of approximately 12 years and a mean frequency of 18 episodes per month. Multiple telangiectases of the hands, face, and oral cavity occur in similar percentages of patients, but the age at onset is generally later than for epistaxis [62,63]. The prevalence of intestinal telangiectasia varies from 10% to 33% [64] in patients with HHT and occur anywhere in the gastrointestinal tract, most commonly in the stomach and upper duodenum. Approximately 25% of individuals older than 60 years present with melena or anemia. Bleeding tends to be slow but persistent and may increase in severity with age [65]. Pulmonary AVMs occur more frequently in patients with HHT1 compared with HHT2—75% and 44%, respectively [66]. They can occur as discrete lesions versus a diffuse pattern and are thought to be congenital and may enlarge over time [67]. They may be asymptomatic for many years and present insidiously or dramatically with respiratory symptoms such as exercise intolerance, cyanosis or pulmonary hemorrhage, migraines, polycythemia, and clubbing [68]. Approximately 30–40% of individuals with HHT who have pulmonary AVMs will have a CNS presentation with thrombotic and embolic events, such as stroke, brain abscess, or transient ischemic attacks, owing to right-to-left shunting that can occur even in the presence of near-normal pulmonary arterial oxygen tension [69]. It is common for several adverse events to occur before a pulmonary AVM is identified as the source of the CNS events [69]. Pregnant women with untreated pulmonary AVMs are at high risk of pulmonary hemorrhage [70]. Cerebral AVMs are thought to be congenital and occur more frequently in individuals with HHT1 compared with HHT2, 15–20% versus 1–2%, respectively [66,71,72]. CNS lesions may present at any age with seizure, headache, or intracranial hemorrhage [73]. In most patients with HHT, liver involvement remains clinically silent, but hepatic vascular lesions (shunts between portal hepatic artery and vein) can present as high-output

Table 56.5 Clinical manifestations and management of patients with hereditary hemorrhagic telangiectasia.

Organ/system	Type of lesion	Sites	Clinical symptoms	Emergencies	Screening tool	Diagnostic modality	Treatment[a]
Nose	Telangiectasia	Nasal mucosa	Epistaxis, iron-deficiency anemia	Massive epistaxis	Medical history	Clinical examination	Humidification, packing, antifibrinolytic therapy (EACA and tranexamic acid), pork salt chop sticks, topical estrogen/progesterone ointments, local cauterization, septal dermoplasty, laser, embolization of external carotid artery, iron therapy
Skin	Telangiectasia	Lips, tongue, palate, face, conjunctivitis, trunk, nail beds, finger pad	Cosmetic disfigurement, bleeding (usually minor)	Nil	Medical history and clinical examination	Clinical examination	Topical agents, laser ablation
Lung	AVM	Often multiple; predilection for lower lobes	Asymptomatic, cyanosis, clubbing, migraine, cerebral abscess, embolic stroke, polycythemia, pulmonary hypertension	Massive hemoptysis, hypovolumic shock, hemothorax	Medical history, auscultation of bruit over chest, blood gas measurement, orthodeoxia[b], pulse oximetry, chest X-ray	High-resolution helical CT scan, angiography	Transcatheter or stereotactic embolization of AVM, surgical resection of AVM, ligation of arterial supply of AVF, iron therapy Note: Require infective endocarditis prophylaxis prior to dental and surgical intervention to reduce the risk of brain abscess
Central nervous system	AVM	Brain, spinal cord, meninges	Asymptomatic, headache, subarachnoid hemorrhage	TIA/ischemic stroke, hemorrhagic stroke, brain abscess	Medical history, auscultation of bruit over the skull	MRI/MRV/MRA/CT scan	Neurovascular surgery, ligation of the feeding arery, stereotactic surgery, transcatheter embolization of the feeding artery, radiosurgery
Gastrointestinal tract (except liver)	AVM, telangiectases, angiodysplasias	Stomach, duodenum, small bowel, colon	Asymptomatic, bleeding, iron deficiency anemia	Hematemesis, melena, hematochezia, hypovolumic shock, high output failure	Medical history, stool guiac examination for the occult blood	Endoscopy, angiography; CT scan	Blood transfusion, endoscopic application of photocoagulation, ethinyl estradiol/norethindrone, iron therapy for anemia
Hepatic	AVM, diffuse telangiectases	Liver parenchyma	Asymptomatic, portal hypertension, biliary disease	Hepatic encephalopathy	Medical history, auscultation of bruit over the liver, ultrasound color Doppler studies	CT scan, MRI/MRV/MRA angiography	No intervention, liver transplantation only for life-threatening lesions

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[a]In life-threatening emergencies ABC should be established first.

[b]Greatest deoxygenation occurs in upright position.

AVM, arteriovenous malformation; CT, computed tomography, EACA, ε-aminocaproic acid.

heart failure, portal hypertension, biliary disease, and portosystemic encephalopathy [74].

AVMs have been described only rarely in other locations, including coronary arteries [75,76] and the vessels of the eye [77], spleen [78], urinary tract [78], and vagina [79].

Diagnosis

The initial diagnosis of HHT in a family relies on clinical examination, medical history, and careful family history [53]. *De novo* mutations are rare and penetrance approaches 100% by the age of 40 years; hence, family history is critical. The diagnosis of HHT in children may be particularly difficult, especially if not previously established in the family.

Based on the multisystem nature of this disease, the initial evaluation once the diagnosis of HHT is established should include the following:

1 chest radiography, arterial blood gas measurements, finger oximetry, and contrast echocardiography remain important in screening persons with suspected pulmonary arteriovenous malformations. Contrast echocardiography to screen for intrapulmonary shunts and, if identified, computed tomography (CT) of the chest with 3-mm cuts to characterize pulmonary AVMs;

2 magnetic resonance imaging of the brain to screen for cerebral AVMs;

3 auscultation for hepatic bruit and medical history for symptomatic liver shunts; and

4 pulmonary angiography is required to plan treatments by interventional radiology or surgery when pulmonary AVMs requiring treatment are identified.

The role of molecular testing for the diagnosis of HHT is limited because of the lack of common alleles or highly recurrent mutations, and locus heterogeneity. Furthermore, the presence of mutations in almost all coding exons of the two genes makes screening for mutations time-consuming and costly. In general, an individual who meets the clinical diagnostic criteria for HHT should be tested first in each family to determine whether the family's HHT mutation is identifiable. Genetic testing for HHT in relatives is not helpful unless a definitive mutation is detected in a clearly affected individual.

Management

In general, treatment of HHT is aimed at (i) control of local and systemic symptoms, (ii) surveillance for and of lesions, and (iii) measures to prevent complications associated with AVMs.

Multiple treatments for epistaxis have been employed, including cauterization, septal dermatoplasty, laser ablation, estrogen therapy, and transcatheter embolization of arteries leading to the nasal mucosa. Antifibrinolytic therapy has been used for the treatment of bleeding in this disorder [80].

Those with a pulmonary arteriovenous malformation should receive antibacterial prophylaxis at the time of dental or surgical procedures. Others who are affected or at risk and who have a family history of pulmonary arteriovenous malformations should use prophylaxis until the possibility that such a malformation is ruled out. Pulmonary AVMs of more than 3 mm in size require intervention. Management of extensive pulmonary AVMs has evolved from lobectomy to wedge resection to ligation of the arterial supply of the malformation. Transcatheter embolotherapy with detachable balloons or stainless-steel coils has also been used to close such malformations. Long-term follow-up of treated patients is important, as the interval growth of malformations may require subsequent intervention. In general, hepatic or CNS AVMs do not require intervention unless they are symptomatic.

It is important that persons with HHT be aware of their diagnosis and its implications; all healthcare providers involved in their care should be informed about the diagnosis. Educational materials for patients and providers are available from the HHT Foundation International (www.hht.org).

Conclusion

Miscellaneous rare bleeding disorders are a group of heterogeneous disorders that may present with a wide range of clinical bleeding symptoms from mucocutaneous bleeding, bleeding after hemostatic challenge such as surgery, menorrhagia, bleeding with labor and delivery, and even catastrophic life-threatening hemorrhage. Diagnosis of these disorders requires careful clinical evaluation, detailed family history, knowledge of the disorders and their associated presentation and sequelae, and specific tests to substantiate the diagnosis. Quite often, immediate and accurate laboratory diagnosis may not be possible because of availability or limitations of diagnostic testing as is exemplified by PAI-1 assays. Hence, a high index of suspicion is required to provide optimal medical care. Therapeutic interventions should be administered only after careful consideration of the risk–benefit ratio and not merely to treat an abnormal laboratory result. In the majority of instances, judicious use of hemostatic agents such as DDAVP, antifibrinolytic therapy, and rFVIIa can control bleeding symptoms. On rare occasions where catastrophic or refractory bleeding is encountered, blood component support may be required. Therapeutic interventions should be tailored to the specific deficiency. For example, antifibrinolytics are utilized as primary hemostatic agents in PAI-1 and α_2-PI deficiency, whereas in specific inherited severe platelet function disorders platelet transfusions can be life-saving. New agents such as NovoSeven® offer an increased range of therapeutic options for these rare disorders in the absence of specific replacement products. The off-label use of rFVIIa as a pan-hemostatic agent has been increasingly described, including use in platelet function disorders. However, consideration of potential adverse events including thromboembolism should be kept in

mind and, therefore, limiting its use to appropriate circumstances is prudent. Patients affected with rare disorders should, whenever possible, be reported to national and international registries specific for the underlying disorder so as to accumulate much needed information that better defines the incidence, clinical manifestations, outcome of treatment, and long-term safety and efficacy of hemostatic agent,s including NovoSeven®.

Resources

- North American rare bleeding disorders registry: www.haemophilia-forum.org/
- International rare bleeding disorders registry: www.hht.org; www.eds.org

References

1 Collen D, Lijnen HR. Basic and clinical aspects of fibrinolysis and thrombolysis. *Blood* 1991; **78**: 3114–24.
2 Rijken DC. Plasminogen activators and plasminogen activator inhibitors: biochemical aspects. *Baillieres Clin Haematol* 1995; **8**: 291–312.
3 Ny T, Sawdey M, Lawrence D, Millan JL, Loskutoff DJ. Cloning and sequence of a cDNA coding for the human beta-migrating endothelial-cell-type plasminogen activator inhibitor. *Proc Natl Acad Sci USA* 1986; **83**: 6776–80.
4 Alessi MC, Juhan-Vague I, Declerck PJ, Anfosso F, Gueunoun E, Collen D. Correlations between t-PA and PAI-1 antigen and activity and t-PA/PAI-1 complexes in plasma of control subjects and of patients with increased t-PA or PAI-1 levels. *Thromb Res* 1990; **60**: 509–16.
5 Rydzewski A, Urano T, Nagai N, *et al*. Diurnal variation in serum remnant-like lipoproteins, platelet aggregation and fibrinolysis in healthy volunteers. *Haemostasis* 1997; **27**: 305–14.
6 Wiman B, Almquist A, Sigurdardottir O, Lindahl T. Plasminogen activator inhibitor 1 (PAI) is bound to vitronectin in plasma. *FEBS Lett* 1988; **242**: 125–8.
7 Lindahl TL, Ohlsson PI, Wiman B. The mechanism of the reaction between human plasminogen-activator inhibitor 1 and tissue plasminogen activator. *Biochem J* 1990; **265**: 109–13.
8 Fay WP, Parker AC, Condrey LR, Shapiro AD. Human plasminogen activator inhibitor-1 (PAI-1) deficiency: characterization of a large kindred with a null mutation in the PAI-1 gene. *Blood* 1997; **90**: 204–8.
9 Lee MH, Vosburgh E, Anderson K, McDonagh J. Deficiency of plasma plasminogen activator inhibitor 1 results in hyperfibrinolytic bleeding. *Blood* 1993; **81**: 2357–62.
10 Pannekoek H, van Meijer M, Schleef RR, Loskutoff DJ, Barbas CF, 3rd. Functional display of human plasminogen-activator inhibitor 1 (PAI-1) on phages: novel perspectives for structure-function analysis by error-prone DNA synthesis. *Gene* 1993; **128**: 135–40.
11 Schleef RR, Higgins DL, Pillemer E, Levitt LJ. Bleeding diathesis due to decreased functional activity of type 1 plasminogen activator inhibitor. *J Clin Invest* 1989; **83**: 1747–52.
12 Minowa H, Takahashi Y, Tanaka T, *et al*. Four cases of bleeding diathesis in children due to congenital plasminogen activator inhibitor-1 deficiency. *Haemostasis* 1999; **29**: 286–91.
13 Rijken DC, Hoegee-de Nobel E, Jie AF, Atsma DE, Schalij MJ, Nieuwenhuizen W. Development of a new test for the global fibrinolytic capacity in whole blood. *J Thromb Haemost* 2008; **6**: 151–7.
14 Holmes WE, Nelles L, Lijnen HR, Collen D. Primary structure of human alpha 2-antiplasmin, a serine protease inhibitor (serpin). *J Biol Chem* 1987; **262**: 1659–64.
15 Bangert K, Johnsen AH, Christensen U, Thorsen S. Different N-terminal forms of alpha 2-plasmin inhibitor in human plasma. *Biochem J* 1993; **291**: 623–5.
16 Koyama T, Koike Y, Toyota S, Miyagi F, Suzuki N, Aoki N. Different NH2-terminal form with 12 additional residues of alpha 2-plasmin inhibitor from human plasma and culture media of Hep G2 cells. *Biochem Biophys Res Commun* 1994; **200**: 417–22.
17 Wiman B, Lijnen HR, Collen D. On the specific interaction between the lysine-binding sites in plasmin and complementary sites in alpha2-antiplasmin and in fibrinogen. *Biochim Biophys Acta* 1979; **579**: 142–54.
18 Moroi M, Aoki N. Inhibition of plasminogen binding to fibrin by alpha2-plasmin inhibitor. *Thromb Res* 1977; **10**: 851–6.
19 Mui PT, James HL, Ganguly P. Isolation and properties of a low molecular weight antiplasmin of human blood platelets and serum. *Br J Haematol* 1975; **29**: 627–37.
20 Collen D, Wiman B. Turnover of antiplasmin, the fast-acting plasmin inhibitor of plasma. *Blood* 1979; **53**: 313–24.
21 Koie K, Kamiya T, Ogata K, Takamatsu J. Alpha2-plasmin-inhibitor deficiency (Miyasato disease). *Lancet* 1978; **2**: 1334–6.
22 Yoshioka A, Kamitsuji H, Takase T, *et al*. Congenital deficiency of alpha 2-plasmin inhibitor in three sisters. *Haemostasis* 1982; **11**: 176–84.
23 Devaussuzenet VM, Ducou-le-Pointe HA, Doco AM, Mary PM, Montagne JR, Favier R. A case of intramedullary haematoma associated with congenital alpha2-plasmin inhibitor deficiency. *Pediatr Radiol* 1998; **28**: 978–80.
24 Takahashi Y, Tanaka T, Nakajima N, *et al*. Intramedullary multiple hematomas in siblings with congenital alpha-2-plasmin inhibitor deficiency: orthopedic surgery with protection by tranexamic acid. *Haemostasis* 1991; **21**: 321–7.
25 Griffin GC, Mammen EF, Sokol RJ, Perrotta AL, Stoyanovich A, Abildgaard CF. Alpha 2-antiplasmin deficiency. An overlooked cause of hemorrhage. *Am J Pediatr Hematol Oncol* 1993; **15**: 328–30.
26 Kordich L, Feldman L, Porterie P, Lago O. Severe hemorrhagic tendency in heterozygous alpha 2-antiplasmin deficiency. *Thromb Res* 1985; **40**: 645–51.
27 Ikematsu S, Fukutake K, Aoki N. Heterozygote for plasmin inhibitor deficiency developing hemorrhagic tendency with advancing age. *Thromb Res* 1996; **82**: 129–16.
28 Kluft C, Vellenga E, Brommer EJ, Wijngaards G. A familial hemorrhagic diathesis in a Dutch family: an inherited deficiency of alpha 2-antiplasmin. *Blood* 1982; **59**: 1169–80.
29 Roche eSMB, WT; Rosenfeld, G. Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. *Am J Physiol* 1949; **150**: 261.
30 Schmaier AH. Plasma kallikrein/kinin system: a revised hypothesis for its activation and its physiologic contributions. *Curr Opin Hematol* 2000; **7**: 261–5.
31 Colman RW, Schmaier AH. Contact system: a vascular biology modulator with anticoagulant, profibrinolytic, antiadhesive, and proinflammatory attributes. *Blood* 1997; **90**: 3819–43.
32 Samlaska CP, James WD, Simel DL. Superficial migratory thrombophlebitis and factor XII deficiency. *J Am Acad Dermatol* 1990; **22**: 939–43.

33 Rao AK. Congenital disorders of platelet function: disorders of signal transduction and secretion. *Am J Med Sci* 1998; **316**: 69–76.
34 George JN. Platelets. *Lancet* 2000; **355**: 1531–9.
35 Mannucci PM. Hemostatic drugs. *N Engl J Med* 1998; **339**: 245–53.
36 Pyeritz RE. Ehlers–Danlos syndrome. *N Engl J Med* 2000; **342**: 730–2.
37 Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers–Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). *Am J Med Genet* 1998; **77**: 31–7.
38 Superti-Furga A, Steinmann B. Impaired secretion of type III procollagen in Ehlers–Danlos syndrome type IV fibroblasts: correction of the defect by incubation at reduced temperature and demonstration of subtle alterations in the triple-helical region of the molecule. *Biochem Biophys Res Commun* 1988; **150**: 140–7.
39 Limongi MZ, Pelliccia F, Rocchi A. Assignment of the human nebulin gene (NEB) to chromosome band 2q24.2 and the alpha 1 (III) collagen gene (COL3A1) to chromosome band 2q32.2 by in situ hybridization; the FRA2G common fragile site lies between the two genes in the 2q31 band. *Cytogenet Cell Genet* 1997; **77**: 259–60.
40 Byers PH. Inherited disorders of collagen gene structure and expression. *Am J Med Genet* 1989; **34**: 72–80.
41 Byers PH, Holbrook KA, McGillivray B, MacLeod PM, Lowry RB. Clinical and ultrastructural heterogeneity of type IV Ehlers–Danlos syndrome. *Hum Genet* 1979; **47**: 141–50.
42 Germain D. Ehlers–Danlos syndromes. Clinical, genetic and molecular aspects. *Ann Dermatol Venereol* 1995; **122**: 187–204.
43 Pepin M, Schwarze U, Superti-Furga A, Byers PH. Clinical and genetic features of Ehlers–Danlos syndrome type IV, the vascular type. *N Engl J Med* 2000; **342**: 673–80.
44 Rudd NL, Nimrod C, Holbrook KA, Byers PH. Pregnancy complications in type IV Ehlers–Danlos syndrome. *Lancet* 1983; **1**: 50–3.
45 Byard RW, Keeley FW, Smith CR. Type IV Ehlers–Danlos syndrome presenting as sudden infant death. *Am J Clin Pathol* 1990; **93**: 579–82.
46 Grenko RT, Burns SL, Golden EA, Byers PH, Bovill EG. Type IV Ehlers–Danlos syndrome with aspirin sensitivity. A family study. *Arch Pathol Lab Med* 1993; **117**: 989–92.
47 Dalgleish R. The Human Collagen Mutation Database 1998. *Nucleic Acids Res* 1998; **26**: 253–5.
48 Bolton-Maggs PH, Perry DJ, Chalmers EA, *et al.* The rare coagulation disorders—review with guidelines for management from the United Kingdom Haemophilia Centre Doctors' Organisation. *Haemophilia* 2004; **10**: 593–628.
49 Stine KC, Becton DL. DDAVP therapy controls bleeding in Ehlers–Danlos syndrome. *J Pediatr Hematol Oncol* 1997; **19**: 156–8.
50 Peery WH. Clinical spectrum of hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu disease). *Am J Med* 1987; **82**: 989–97.
51 Guttmacher AE, Marchuk DA, White RI, Jr. Hereditary hemorrhagic telangiectasia.[see comment]. *N Engl J Med* 1995; **333**: 918–24.
52 Shovlin CL, Hughes JM. Hereditary hemorrhagic telangiectasia. *N Engl J Med* 1996; **334**: 330–1; author reply 1–2.
53 Kjeldsen AD, Vase P, Green A. Hereditary haemorrhagic telangiectasia: a population-based study of prevalence and mortality in Danish patients. *J Intern Med* 1999; **245**: 31–9.
54 ten Dijke P, Hill CS. New insights into TGF-beta-Smad signalling. *Trends Biochem Sci* 2004; **29**: 265–73.
55 Wrana JL, Attisano L, Wieser R, Ventura F, Massague J. Mechanism of activation of the TGF-beta receptor. *Nature* 1994; **370**: 341–7.
56 Schmierer B, Hill CS, Schmierer B, Hill CS. TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility. *Nat Rev Mol Cell Biol* 2007; **8**: 970–82.
57 McDonald MT, Papenberg KA, Ghosh S, *et al.* A disease locus for hereditary haemorrhagic telangiectasia maps to chromosome 9q33–34. *Nat Genet* 1994; **6**: 197–204.
58 Shovlin CL, Hughes JM, Tuddenham EG, *et al.* A gene for hereditary haemorrhagic telangiectasia maps to chromosome 9q3. *Nat Genet* 1994; **6**: 205–9.
59 Johnson DW, Berg JN, Gallione CJ, *et al.* A second locus for hereditary hemorrhagic telangiectasia maps to chromosome 12. *Genome Res* 1995; **5**: 21–8.
60 Cole SG, Begbie ME, Wallace GM, Shovlin CL. A new locus for hereditary haemorrhagic telangiectasia (HHT3) maps to chromosome 5. *J Med Genet* 2005; **42**: 577–82.
61 Bayrak-Toydemir P, McDonald J, Akarsu N, *et al.* A fourth locus for hereditary hemorrhagic telangiectasia maps to chromosome 7. *Am J Med Genet* 2006; **140**: 2155–62.
62 Plauchu H, de Chadarevian JP, Bideau A, Robert JM. Age-related clinical profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population. *Am J Med Genet* 1989; **32**: 291–7.
63 Porteous ME, Burn J, Proctor SJ. Hereditary haemorrhagic telangiectasia: a clinical analysis. *J Med Genet* 1992; **29**: 527–30.
64 Ingrosso M, Sabba C, Pisani A, *et al.* Evidence of small-bowel involvement in hereditary hemorrhagic telangiectasia: a capsule-endoscopic study. *Endoscopy* 2004; **36**: 1074–9.
65 Kjeldsen AD, Kjeldsen J. Gastrointestinal bleeding in patients with hereditary hemorrhagic telangiectasia. *Am J Gastroenterol* 2000; **95**: 415–18.
66 Sabba C, Pasculli G, Lenato GM, *et al.* Hereditary hemorrhagic telangiectasia: clinical features in ENG and ALK1 mutation carriers. *J Thromb Haemost* 2007; **5**: 1149–57.
67 Vase P, Holm M, Arendrup H. Pulmonary arteriovenous fistulas in hereditary hemorrhagic telangiectasia. *Acta Med Scand* 1985; **218**: 105–9.
68 Shovlin CL, Letarte M. Hereditary haemorrhagic telangiectasia and pulmonary arteriovenous malformations: issues in clinical management and review of pathogenic mechanisms. *Thorax* 1999; **54**: 714–29.
69 White RI, Jr., Pollak JS, Wirth JA. Pulmonary arteriovenous malformations: diagnosis and transcatheter embolotherapy. *J Vasc Interv Radiol* 1996; **7**: 787–804.
70 Shovlin CL, Winstock AR, Peters AM, Jackson JE, Hughes JM. Medical complications of pregnancy in hereditary haemorrhagic telangiectasia. *QJM* 1995; **88**: 879–87.
71 Bossler AD, Richards J, George C, Godmilow L, Ganguly A. Novel mutations in ENG and ACVRL1 identified in a series of 200 individuals undergoing clinical genetic testing for hereditary hemorrhagic telangiectasia (HHT): correlation of genotype with phenotype. *Hum Mutat* 2006; **27**: 667–75.
72 Fulbright RK, Chaloupka JC, Putman CM, *et al.* MR of hereditary hemorrhagic telangiectasia: prevalence and spectrum of cerebrovascular malformations. *AJNR Am J Neuroradiol* 1998; **19**: 477–84.
73 Matsubara S, Mandzia JL, ter Brugge K, Willinsky RA, Faughnan ME. Angiographic and clinical characteristics of patients with cerebral arteriovenous malformations associated with hereditary

hemorrhagic telangiectasia. *AJNR Am J Neuroradiol* 2000; **21**: 1016–20.

74 Memeo M, Stabile Ianora AA, Scardapane A, Buonamico P, Sabba C, Angelelli G. Hepatic involvement in hereditary hemorrhagic telangiectasia: CT findings. *Abdom Imaging* 2004; **29**: 211–20.

75 Aguilera B, Suarez-Mier MP, Argente T. Cardiac arteriovenous malformation causing sudden death. *Cardiovasc Pathol* 2004; **13**: 296–8.

76 Hsi DH, Ryan GF, Hellems SO, Cheeran DC, Sheils LA. Large aneurysms of the ascending aorta and major coronary arteries in a patient with hereditary hemorrhagic telangiectasia. *Mayo Clin Proc* 2003; **78**: 774–6.

77 Vase I, Vase P. Ocular lesions in hereditary haemorrhagic telangiectasia. *Acta Ophthalmol (Copenh)* 1979; **57**: 1084–90.

78 Kato S, Ichihara K. Hereditary hemorrhagic telangiectasia with malignant lymphoma. An autopsy case. *Acta Pathol Jpn* 1988; **38**: 383–91.

79 Humphries JE, Frierson HF, Jr., Underwood PB, Jr. Vaginal telangiectasias: unusual presentation of the Osler–Weber–Rendu syndrome. *Obstet Gynecol* 1993; **81**: 865–6.

80 Saba HI, Morelli GA, Logrono LA. Treatment of bleeding in hereditary hemorrhagic telangiectasia with aminocaproic acid. *N Engl J Med* 1994; **330**: 1789–90.

57 Emergency management of hemophilia

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Patients with hemophilia A or B of all severities are at increased bleeding risk following injury of any origin. Even when prophylaxis is being employed to reduce or eliminate hemarthroses in patients with severe or moderately severe hemophilia, a significant portion of an individual hemophilic patient's daily living is occurring with subphysiologic plasma levels of his missing clotting factor. Should he incur a major injury during these periods of nonoptimal levels of factor VIII or IX, he is at increased risk for life- or limb-threatening bleeding, at a minimum analogous to that of a patient with mild hemophilia having a comparable injury. Hence, therapeutic strategies for treating life- or limb-threatening injuries follow common principles regardless of severity or whether the patient has hemophilia A or B. This chapter will review these therapies for injuries to vital organs and provide a guidance concerning key therapeutic issues such as duration of clotting factor replacement and other acute care management strategies.

Central nervous system bleeding

The most frequently encountered life-threatening event among the hemophilia population is intracranial hemorrhage. Supporting this conclusion is a recent report concerning inhibitor mortality reduction among patients with high-titer inhibitors reported from the UK Haemophilia Centre Doctors' Organisation database during the last decade of the twentieth century compared with the death rates for the aggregate hemophilia population during the prior two decades [1]. Trauma is the inciting event for severe central nervous system (CNS) hemorrhage (intracranial or paraspinal) in hemophilia patients most commonly. However, it is well recognized that slowly evolving hemorrhages or hematomas that begin as inconsequential can be followed by recurrent bleeding in the area of the initial bleeding leading to a much greater neurologic impairment. Therefore, a bleed thought to have occurred spontaneously may well reflect this scenario rather than a *de novo* event [2,3]. Stated another way, spontaneous CNS bleeding in hemophilia, even among patients with a severe phenotype, appears to be rare. Exceptions may indicate the presence of an anatomic lesion, such as an arteriovenous malformation or aneurysm. Besides trauma, other predisposing risk factors for intracranial hemorrhage include HIV infection accompanied by immune suppression, the presence of an inhibitor, and age <5 years or >51 years [3]. All severities of hemophilia are at increased risk for CNS hemorrhage compared with the normal population and even patients with >5% factor VIII or IX have an increased risk approaching 50% of those with severe disease.

Intracranial hemorrhage

Compression from an increasing volume of blood inside the calvarium or spinal canal can rapidly induce a compression injury of the vulnerable neurons and glial cells. Depending on the location of the bleed, even small volumes of blood can induce such injury because of markedly constrained expansion capacity in these anatomic sites. Therefore, from a therapeutic standpoint, it is critical that a physiologic hemostatic potential through infusion of the missing factor be undertaken immediately when such a bleed is suspected—even before confirming the presence of the bleed with diagnostic imaging.

Intracranial hemorrhage (ICH) can occur in the subdural space, the epidural/subarachnoid space, or the intraparenchymal tissues [4]. Bleeding at any of these three sites can cause rapid progression of symptomatology such as headache, lateralizing neurologic dysfunction, or even acute-onset loss of consciousness. This results not only from the mass effect of the blood itself but also the accompanying inflammation which induces brain swelling (Tables 57.1 and 57.2) [5]. The latter, in the worst case scenario, can lead to herniation of the brainstem and death. Conversely, rapid replacement with clotting factor concentrates may forestall most or all neurologic sequelae, as long as the restoration of physiologic hemostasis is of sufficient duration to allow CNS vessel healing to occur. Even in the latter scenario, the risk for rebleeding at a proximal CNS site may always be higher than prior to the original hemorrhage [6–10]. Further, intraparenchymal hemorrhages in particular can cause symptoms out of proportion to the size of the amount of hemorrhage [11], particularly in young children with hemophilia. This is particularly true when bleeding occurs

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Table 57.1 High-risk hemorrhagic events for acute morbidity or mortality in hemophilia A or B [5].

Central nervous system hemorrhage
Intracranial hemorrhage
Paraspinal hemorrhage
Soft-tissue hemorrhage predisposing to airway impingement
Retropharyngeal hemorrhage following mandibular molar extraction leading to hemorrhage along facial planes
Neck hematoma associated with dissection
Tracheal hemorrhage following airway instrumentation
Large tongue hematoma
Gastrointestinal bleeding
Hematemesis from esophageal injury or ulceration of gastric or duodenal mucosa
Hemorrhage from ruptured esophageal varix (varices)
Hematochezia or melena from bleeding telangiectasia polyps, etc.
Ruptured abdominal organ or capusular hematoma of abdominal viscus
Splenic rupture, kidney capsular rupture, liver laceration
Hematoma of bowel wall
Ruptured appendix
Ruptured pelvic or abdominal pseudotumor
Acute compartment syndrome
Hematoma impingement of nerves, vasculature of extremities
Hemorrhage in or around the eye
Hyphema
Vitreous hemorrhage
Hematoma following orbital fracture

Table 57.2 Guidelines for acute management of severe hemorrhage in hemophilia A and B [5].

Assure adequate airway, breathing, and circulation by assessing respirations, pulse, and blood pressure (basic cardiopulmonary resuscitation guidelines)
Attain venous access as expeditiously as possible
Infuse appropriate factor VIIII (hemophilia A) or factor IX (hemophilia B) at a dose calculated to achieve physiologic levels immediately [50 U/kg body weight factor VIII or 100–120 U/kg high-purity factor IX (70–80 U/kg of prothrombin complex concentrate if high-purity factor IX is unavailable), respectively]
Obtain computed tomography scan, ultrasound, or other imaging studies as indicated to ascertain bleeding site/source
Request consultation from appropriate physician consultant for bleeding site, e.g., ophthalmologist for bleeding in/around the eye
Hospitalize
Monitor factor VIII/IX levels (for hemophilia A/B, respectively) on a frequent basis to maintain level in the mid-physiologic range
Continue with frequent bolus or continuous clotting factor infusions adjusted according to measured factor VIII/IX plasma levels until the acute bleeding event has resolved. Factor VIII/IX dosing may be adjusted downward as the risk for further bleeding is substantially reduced
Examine the patient following hospitalization to ensure that any sequelae receive appropriate long-term care

at a brain locus essential for maintenance of vital organ functioning such as the brainstem.

Since clinical outcome can be related to both the volume of brain injured and the duration of the bleeding-associated inflammation (particularly morbid brain swelling), neurosurgical intervention to evacuate the hemorrhage may save the life of the person with hemophilia and/or preserve his or her long-term neurologic function [10]. This, of course, requires adequate hemostasis during and following the surgical intervention with sufficient clotting factor replacement (or bypassing activity in the case of a patient with a high-titer inhibitor). Strategies for insuring the adequacy of the hemostasis will be discussed in a following section (Clotting Factor Replacement). For comatose or incapacitated patients, airway protection, appropriate cardiovascular monitoring, and careful maintenance of physiologic intracranial pressure are requisites for an optimal outcome [12,13].

Neonatal central nervous system hemorrhage

Intracranial hemorrhage in the newborn occurs most commonly following birth trauma [11]. The use of forceps delivery or vacuum extraction to "facilitate" difficult vaginal delivery is particularly prone to result in ICH among hemophilic neonates [14]. Kulkarni *et al.*, in the largest review to date, cite an ICH incidence of 3.58% [15]. Because approximately 30% of male infants with hemophilia represent index cases in a family (because of *de novo* mutations), all males presenting at birth with diagnosed ICH should be suspected of having a congenital bleeding disorder such as hemophilia A or B. Confirmation with transfontanelle ultrasonography should be done immediately. Concurrently, the neonate should be assessed with a coagulation work-up that includes, minimally, a prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet count. If the aPTT alone is abnormal, a factor VIII (followed by factor IX assay should the former be normal) should be performed emergently to direct appropriate replacement therapy [16,17].

Spinal hematoma

Even though the treatment of choice for blood-induced spinal compression is neurosurgical decompression, in patients with hemophilia rapid infusion of clotting factor replacement at the first symptom of pain may obviate the need for surgery. Rapid stanching of bleeding may prevent progression to sensory and motor nerve compromise [18]. In addition, repletion of the missing clotting factor may prevent later spinal deformities when such bleeds occur in young children [19]. In some instances, when unequivocal neural compromise is present, a laminectomy to decompress the cord may be unavoidable. In the latter scenario, a carefully monitored clotting factor replacement strategy, similar to that required for neurosurgery, to treat ICH is mandatory. This treatment should

continue for a minimum of 10–14 days, even when laminectomy is avoidable [20]. As with ICH, suspicion that bleeding is compressing the spinal cord requires rapid clotting factor replacement, even before imaging studies to confirm the morbidity are undertaken. Once imaging studies are performed, which verify the presence of blood in the spinal canal, the hemophilic patient needs to be admitted to a critical care unit to insure that hemostasis is adequate for the required duration—even when symptoms rapidly remit.

Clotting factor replacement: recommendations for the treatment of central nervous system bleeds

Three basic principles define strategies for adequate hemostasis replacement therapy for central nervous system bleeding:
1 Infuse enough factor VIII or IX concentrates for hemophilia A or B, respectively, to achieve a measured recovery of approximately 100%. For patients with inhibitors (discussed in greater detail in Chapters 9, 10 and 11), dosing is less precise but should aim for the maximum demonstrated safe dose for the respective bypassing agent.
2 Infuse FVIII or IX often enough to insure that nadir levels are at the low end of physiologic levels (50% minimum for FVIII, perhaps slightly lower for FIX if the patient is older and felt to be at increased risk of deep vein thrombosis).
3 Obtain *in vivo* measurements of FVIII or IX levels as frequently as required to ensure that these minimal physiologic levels are consistently achieved.

In patients with hemophilia A, a dose of 50 U/kg factor VIII initially then every 8–12 h will typically achieve these levels in a patient with severe disease. If surgery is required, the second dose may need to be given earlier than this because clearance is typically greater because of perioperative hemostatic requirements. Once the clearance has returned to steady state (which may occur after the initial dosing when surgery is not needed), both less frequent and lower dosing may be needed to keep the levels in the physiologic range. There is *no* substitute for measuring the factor VIII level to ascertain how to appropriately adjust dosing.

For patients with hemophilia B, dosing will likely need to be product-type specific. When recombinant factor IX is infused, an initial dose of 120 U/kg followed by adjusted dosing based on whether or not surgery is needed (see above, Inhibitors to factor VIII, Chapters 9, 10 and 11). Once clearance is close to steady state, dosing frequency will likely need to be every 18–24 h [5]. If high-purity plasma-derived concentrates are administered, a loading dose of approximately 100 U/kg followed by a similar factor IX level directed follow-up dosing schedule as with recombinant may be employed. Plasma-derived prothrombin complex concentrates (PCCs) should not be used for managing these patients if either of the above concentrates are available because of a demonstrably greater risk for excess clotting and even disseminated intravascular coagulation. If a PCC is all that is available, an initial dose of 80–100 U/kg is reasonable, but subsequent dosing should be reduced compared with the above recommendations because of the thrombotic risks. Data using these products suggest dosing to achieve nadir levels of 30–40% may provide a safe window that balances bleeding and thrombotic risks [21]. In addition to monitoring factor IX levels, platelet count, PT, aPTT, fibrinogen and a fibrinolytic marker such as D-dimer should be monitored when PCCs are used in this context to monitor for any insidious thrombotic potential.

Alternative strategies for providing hemostatic coverage for patients with CNS hemorrhages, including continuous infusion of either factor VIII or IX, are discussed elsewhere (Chapter 6). As with any major surgery or life-threatening event, this alternative has the advantage of more consistent *in vivo* levels but the potential disadvantage that temporary interruption of the infusion (such as might occur with infiltration of the infusion line) may lead to bleeding. These need to be balanced for each individual circumstance.

The duration necessary to achieve an optimal clinical outcome varies with the injury, whether or not neurosurgery is required, and other confounding factors such as whether the patient has had any antecedent CNS bleeding that may indicate a high predisposition for recurrence. Regardless, the propensity for rebleeding to occur following ICH, for example, appears to be significant. This is particularly true if hemostatic replacement is discontinued prematurely [22]. For this reason, many hemophilia experts advocate a long-term prophylaxis regimen for children and adults who have had a large ICH. This strategy is consistent with observations that neovascularization in areas of the brain affected by hemorrhagic stroke may, for quite some time, result in fragile vessels that may bleed with a minimal insult. The duration of any secondary prophylaxis (distinct from that in individuals already on primary prophylaxis who would presumably return to this regimen once the intensive event-associated replacement therapy is complete) will depend on many factors, such as availability of factor concentrates, severity of injury and associated conditions, patient willingness to adhere to the regimen, and rehabilitative regimens [5].

Neurosurgical management of acute central nervous system events in patients with high-titer inhibitors

Appropriate dosing of bypassing agents for major hemorrhagic events or emergency surgery are discussed in Chapters 23 and 24 (FEIBA and rFVIIa). As noted there, an invariably reliable hemostasis for such dire circumstances is problematic when such an antibody against factor VIII or IX is present. Nonetheless, effective hemostasis for both CNS events and the accompanying necessary surgery has been demonstrated in many such circumstances [23]. Establishing a treatment plan prospectively for any high-titer inhibitor patient who may, at some point, incur a brain or spinal cord injury based on his

known responsiveness to either bypassing agent is probably a sound strategy. Further, careful serial monitoring of his inhibitor titer may indicate when, if ever, very large doses of either factor VIII (hemophilia A) or IX (hemophilia B) might be utilized initially for a temporarily low titer. The use of these factors in such circumstances before anamnesis occurs allows one to demonstrate an adequate measurable *in vivo* level for the critical period of time immediately following injury. Once the inevitable anamnesis does occur, bypassing agents can be utilized for the duration of the convalescent period. In rare life-threatening circumstances, a combination of bypassing agents and factor replacement may be required to achieve hemostasis. The risks of combining such agents in such events are justified when all other efforts to stop bleeding has failed.

Non-central nervous system-emergent events

Injury in proximity to the airway

Although CNS injury represents the most commonly observed extreme debility or life-threatening injury state in patients with hemophilia, bleeding that has the potential to lead to airway obstruction may represent the most emergent [16]. Extreme trauma may produce sufficient bleeding to immediately compromise the patency of the trachea. On the other hand, the period from injury to acute airway compromise may be long (hours, or even days). However, the evolution to criticality may then be rapid once the patient begins experiencing dyspnea. This symptom implies significant compromise of the tracheal lumen indicating that further bleeding has the immediate potential to progress to asphyxia. Therefore, hemostatic therapy at this stage may need to be administered concurrently with surgical preparation for tracheostomy. Therefore, there is no substitute for early bleed recognition and rapid replacement to forestall this extreme intervention [24].

Neck injuries or retropharyngeal dissecting hematomas (particularly following molar oral surgery) along facial planes of the neck are the most likely initiating events for tracheal compression from hemorrhage. In rare circumstances, an early sign of a bleeding event with the potential to progress to airway compression is facial swelling [25]. In addition, swelling of the tongue owing to unrecognized injury to the lingual artery may progress to a sufficient mass to block the posterior pharynx [26]. To prevent the risks of oral maxillofacial surgery producing such bleeding in a patient with hemophilia, adequate clotting factor replacement therapy (to normal physiologic levels of approximately 100%) is essential [27]. Accordingly, alternative anesthesia to alveolar nerve block for dental surgery will decrease the likelihood of such events. Clearly, prior consultation between the hemostasis expert and the otolaryngologist is necessary to reduce the risks for morbid bleeding in these circumstances. If airway risks are deemed high or any symptoms of airway compromise are present, anesthesia expertise is also needed.

After the initial dose of clotting factor has been administered, follow-up infusions frequent enough to maintain physiologic levels of factor VIII or IX, respectively, or continuous infusion are required, as is frequent monitoring of the factor VIII/IX level. This should be continued until all neck swelling has resolved and/or the surgical wound shows clear signs of healing. Typically, these life-threatening bleeding episodes will require the patient to be hospitalized in a critical care unit until the risks are controlled and to undergo inpatient hemostatic management for at least 1 week. If the patient was not receiving prophylaxis prior to the injury or surgery, a several-week course of outpatient secondary prophylaxis may be prudent until the wound is completely healed.

Gastrointestinal hemorrhage

Bleeding in hemophilia patients can occur from any gut site from the esophagus to the anus. A review by Mittal *et al*. of 41 episodes of gastrointestinal (GI) hemorrhage in patients with hemophilia cited duodenal ulcer (22%) and gastritis (21%) as the most frequent anatomic sites [28]. Yet, in another 22% no source was identified. In a separate series, Mallory–Weiss syndrome (injury to the mucosa of the esophagus, sometimes related to severe coughing) was cited as a cause of GI hemorrhage in hemophilia patients [29].

When bleeding is from the lower GI tract, as indicated by melena in a patient with hemophilia, clotting factor replacement is almost always required to stop the bleeding, regardless of the source. Accordingly, a vigorous attempt to identify a source using colonoscopy, imaging, or both should follow initial replacement, particularly in adult patients where the risk for colon cancer is not insignificant. Usually when the bleeding is mild or moderate, replacement therapy alone suffices unless an anatomic lesion requiring resection is identified [30]. When the amount of blood loss is copious, providing immediate clotting factor replacement to physiologic levels is crucial, followed when the bleeding fails to stop, with the appropriate diagnostic measures. The needed interventional therapy is directed by a gastroenterologist.

Age considerations are also important. In a neonate, melena or hematochezia may represent the initial bleeding event in a *de novo* presentation of a hemophilia phenotype [31]. By contrast, among adult patients with hemophilia and concomitant hepatitis C infection and cirrhosis, acute bleeding from esophageal varices may be life threatening [32,33]. In this instance, immediate hemostatic replacement therapy may need to be followed by gastroenterologic intervention (e.g., transjugular intrahepatic portosystemic shunt). Once the venous pressure in the varices is reduced by such a procedure, follow-up infusions of physiologic doses of clotting factor will be needed to allow the requisite repair to occur.

On occasion, patients with varices or other manifestations of chronic liver failure will have their hemostatic course

complicated by the accompanying deficiencies of other liver-produced clotting factors. Particularly vulnerable to impaired synthesis are the vitamin K-dependent serine proteases II, VII, IX, and X. When these are superimposed on both the hemophilia (in particular factor VIII deficiency) and the dilated veins, the patient is at very high risk for life-threatening bleeding. In addition to the above therapeutic strategies, replacement of the acquired deficiencies must be undertaken as well. Replacement with either fresh-frozen plasma or prothrombin complex concentrate may be required. The latter must be administered judiciously because of the additional possibility of a concomitant thrombosis risk.

Massive melena rarely has hemophilia as the primary etiology. Vascular lesions such as arteriovenous malformations or bleeding telangiectasias are often the etiologies for such massive blood loss from the lower GI tract [34]. Clotting factor replacement initially allows the necessary interventional diagnostic/therapeutic procedure to be performed, and follow-up infusions are then needed until healing unassociated with occult GI hemorrhage is achieved. The duration will depend also on whether abdominal surgery is required to remove the source of the hemorrhaging.

Bleeding from organ rupture or hematoma of an abdominal viscus

Evolving *intraperitoneal* hemorrhage caused by blunt abdominal trauma and associated with massive blood loss can, particularly among patients with hemophilia, lead to *exsanguination*. Typically, a history of recent trauma is present [34]. Rarely among the hemophilia population, however, are reports of bleeding within a viscus evolving to organ rupture *without demonstrable trauma* [35,36].

It would appear that the circumstances under which this occurs are very rare. However, mild previous traumatic insults may induce low-grade bleeding that becomes recurrent within the confines of the organ. Encapsulated abdominal organs in which this insidious and progressive hemorrhaging have been described include the spleen, liver, kidneys, bowel wall, and pancreas. When the bleeding does not tamponade, the pressure from the expanding hematoma may become sufficiently high to induce rupture of the capsule and extreme acute blood loss [37]. When the event occurs within the wall of the gut there may also be the risk of gut obstruction prior to actual rupture. Abdominal pain (or abdominal distension in neonates) [38] may be the only symptom. Certainly, any history of recent abdominal trauma, no matter how mild, in a patient with hemophilia should arouse suspicion, probably justify immediate infusion and, without quick resolution of the pain, necessitate imaging with ultrasound or computed tomography (CT) to identify the presence of a hematoma.

When the presence of such a hematoma is not suspected or if replacement therapy was of insufficient dose or duration following diagnosis, a real risk for rupture exists. When that happens blood loss can be very large, shock can ensue, and cardiac arrest can follow quickly. Resuscitation with volume expansion, packed red blood cells and factor replacement are required invariably and, in many instances, laparotomy may represent the only mechanism to stanch the bleeding. The latter may be necessary to remove the bleeding organ (e.g., splenectomy) [39,40] or to repair it (liver, kidney, or bowel wall) [35]. Early infusion of clotting factor may pre-empt the expansion of the hematoma and forestall surgery in emergent circumstances [41].

Although hematomas of the psoas and obturator muscles represent fairly common bleeding manifestations among patients with severe hemophilia, rarely is the bleeding sufficient to cause a significant drop in hemoglobin. Typically, the bleeding will tamponade. Nonetheless, bleeding can be significant enough to compress sacral nerves, causing significant pain and immobility [42]. Clotting factor replacement and physical therapy by experts in managing hemophilia patients remain the mainstay of therapy for this bleeding event. Rarely is surgery required unless there is evolution to a pseudotumor (see Chapter 29). (surgical/orthopedic chapter that discusses psuedotumors)

When an individual with hemophilia experiences a rupture of his appendix, it may be difficult to differentiate from pseudohemophilia, which is a small hematoma of the intestinal wall in the area of the right lower quadrant. Either may be associated with no apparent antecedent trauma [43]. Modern imaging techniques should readily distinguish between the two entities [41]. In either instance early replacement therapy is essential, even if abdominal surgery is not contemplated immediately [38].

Symptoms of nerve compression or compartment syndrome

There is a risk for permanent peripheral neurologic debilitation when hemophilic bleeding in a closed anatomic compartment continues until the vascular or neurologic bundles are compromised. This morbidity, occurring in proximal or distal extremities, is known as a "compartment" syndrome and typically is a consequence of either local trauma or iatrogenically induced bleeding. Examples of the latter include aborted attempts at arterial cannulation or venipuncture (particularly among neonates and small infants). Symptoms begin as swelling and engorgement and, if the bleeding continues into the confined space, progress to paresthesia or even paresis, arterial compression, loss of pulse, and a cold extremity.

As with suspected CNS hemorrhage, early recognition followed by aggressive clotting factor replacement can prevent this evolution of morbidity. In those instances when the injured extremity is clearly cooler and paler than the contralateral one, surgical *fasciotomy* may be necessary. However, fasciotomies in patients with hemophilia are fraught with risk for protracted bleeding even when prior replacement therapy

has been given. Therefore, a good strategy is education of patients about the possibility of a compartment syndrome when closed extremity bleeding is progressing. This recognition must be combined with immediate dosing of factor concentrate to achieve a level of approximately 100% of normal. Thereby, most fasciotomies can be avoided.

Ophthalmologic emergencies

When bleeding occurs in a hemophilia patient's eye (usually following facial or head trauma) [44], this is an extremely emergent event. Bleeding into the anterior chamber (hyphema), vitreous humor, or lens (the rare entity known as hemorrhagic glaucoma) [45–47] jeopardizes vision both acutely and, if of significant magnitude, permanently. Sight can also be compromised by a retinal hematoma forming at the site of a retinal detachment. As with many of the emergent strategies discussed above, a parallel strategy of therapeutic intervention is essential: immediate administration of clotting factor sufficient to raise the *in vivo* level to 100% *and* urgent assessment by an experienced ophthalmologist. Hospitalization until the clinical situation stabilizes is necessary even if eye surgery is felt not to be indicated. Following discharge from the hospital, collaborative outpatient management by both the ophthalmologist and the hemophilia physician is essential to insure an optimal outcome.

Rare clinical emergencies

Some bleeding events are considered emergent whether or not there is an underlying bleeding disorder. Examples include pericardial bleeding, which can cause life-ending tamponade, or pulmonary hemorrhage of a severity, which can cause severe respiratory distress. In those rare circumstances when these occur in patients with hemophilia, the management complexity increases. The emergency surgery necessary to save the hemophilic individual's life cannot be permitted to exacerbate the bleeding itself because of insufficient concomitant to treatment of the patient's hemophilia.

Rupture of a pseudotumor

Although the presence of a hemophilic pseudotumor does not constitute a hemostatic emergency, rupture of a large one in the pelvis or thigh can lead to massive hemorrhage and shock [48]. Immediate replacement therapy with sufficient factor VIII or IX, respectively, to an *in vivo* level of 1 U/mL (100%) is, of course, required. However, since pseudotumors typically have large volumes of partially clotted blood that has induced significant fibrinolysis, fibrin degradation products may be released, which slow the rate of new fibrin cross-linking. This can predispose to further bleeding even when the factor VIII/IX levels are normal. Laboratory assessment for such secondary fibrinolysis is therefore indicated. When present, concurrent therapy with a fibrinolytic inhibitor such as ε-aminocaproic acid or tranexamic acid may also be needed. Further, surgical resection/evacuation of the remnants the tumor may be needed for healing to occur.

Conclusion

Life-threatening bleeding is uncommon in patients with hemophilia. When such bleeding occurs it is often the result of an injury that would induce severe bleeding even in an individual without an underlying bleeding disorder. For the hemophilic patient, however, restoration of the plasma level of the missing procoagulant (VIII or IX) to normal is essential in such circumstances so that other necessary medical interventions to treat the injury do not compound the hemorrhagic state. In terms of priority, only the removal of an acutely injurious agent or cardiopulmonary resuscitation take precedent over clotting factor replacement in an injured individual with hemophilia. This is true regardless of the severity of the hemophilia. Further, initial clotting factor replacement must be followed by recurrent dosing or continuous administration until sufficient healing has occurred. Simultaneous laboratory measurement to insure adequate *in vivo* levels should be done if at all possible. There also may be special medical management that is required to treat the specific injury or disease state. For these, the expertise of the appropriate medical specialist should be sought. Knowledge of the specialized hemostatic management required in such scenarios and prior discussion of how to proceed in the respective circumstance can help to assure a good clinical outcome when these daunting events do happen.

References

1 Darby SC, Kan SW, Spooner RJ, *et al.* Mortality rates, life expectancy, and causes of death in people with hemophilia A or B in the United Kingdom who were not infected with HIV. *Blood* 2007; **110**: 815–25.
2 Visconti EB, Hilgartner MW. Recognition and management of central nervous hemorrhage in hemophilia. *Pediatrician* 1980; **9**: 127–37.
3 Federici A, Minetti D, Grande C, Gatti L, Mannucci PM. Intracranial bleeding in haemophilia: A study of eleven cases. *Haematologica* 1982; **67**: 747–53.
4 Nuss R, Soucie JM, Evatt B, and the Hemophilia Surveillance System Project Investigators. Changes in the occurrence of and risk factors for hemophilia-associated intracranial hemorrhage. *Am J Hematol* 2001; **68**: 37–42.
5 Hoots WK. *Emergency Management of Haemophilia*. World Federation of Haemophilia Monograph, 2008.
6 Tseng SH. Delay traumatic intracerebral hemorrhage: a study of prognostic factors. *J Formos Med Assoc* 1992; **91**: 585–9.

7 Mamoli B, Sonneck G, Lechner K. Intracranial and spinal hemorrhage in hemophilia. *J Neurol* 1976; **211**: 143–54.
8 Walike JW, Chinn J. Evaluation and treatment of acute bleeding from the head and neck. *Otolaryngol Clin North Am* 1979; **12**: 455–64.
9 Bentacor N, Lavalle E, Vila VM, Johnston E, Borovich B. Intracranial hemorrhage in hemophiliacs. Study of 10 episodes. *Sangre* 1992; **37**: 43–6.
10 Borthne A, Sortland O, Blikra G. Head injuries with delayed intracranial hemorrhage. *Tidsskr Nor Laegeforen* 1992; **112**: 3425–8.
11 Yoffe G, Buchanan G. Intracranial hemorrhage in newborn and young infants with hemophilia. *J Pediatr* 1988; **113**: 333–6.
12 Martinowitz U, Heim M, Tadmor R, *et al.* Intracranial hemorrhage in patients with hemophilia. *Neurosurgery* 1986; **18**: 538–41.
13 DeBehnke DJ, Angelos MG. Intracranial hemorrhage and hemophilia: case report and management guidelines. *J Emerg Med* 1990; **8**: 423–7.
14 Ohga S, Kajiwara M, Toubo Y, *et al.* Neonatal hemophilia B with intracranial hemorrhage. *Am J Pediatr Hematol Oncol* 1988; **10**: 244–8.
15 Kulkarni R, Lusher JM. Intracranial and extracranial hemorrhages in newborns with hemophilia: a review of the literature. *J Pediatri Hematol Oncol* 1999; **21**: 289–95.
16 Bray GL, Luban NLC. Hemophilia presenting with intracranial hemorrhage. *AJCD* 1987; **141**: 1215–17.
17 Pettersson H, McClure P, Fitz C. Intracranial hemorrhage in hemophilic children. *ACTA Radiol* 1984; **25**: 161–4.
18 Myles LM, Massicotte P, Drake J. Intracranial hemorrhage in neonates with unrecognized hemophilia A: a persisting problem. *Pediatr Neurosurg* 2001; **34**: 94–7.
19 Yasouka S, Peterson HA, McCarty CS. Incidence of spinal column deformity after multi level laminectomy in children and adults. *J Neurosurg* 1982; **57**: 441–5.
20 Narawong D, Gibbons VP, McLaughlin JR, Bouhasin JD, Kotagal S. Conservative management of spinal epidural hematoma in hemophilia. *Pediatr Neurol* 1988; **4**: 169–71.
21 Blatt PM, Goldsmith JC, Roberts HM. Prothrombin complex concentrates. *Arch Intern Med* 1978; **138**: 827.
22 Eyster ME, Gill FM, Blatt PM, Hilgartner MW, Ballard JD, McKinney TR. Central nervous system bleeding hemophiliacs. *Blood* 1978; **51**: 1179–88.
23 Negrier C, Hay CM. The treatment of bleeding in hemophilia patients with inhibitors with recombinant Factor VIIa. *Seminar Thromb Hemost* 2000; **26**: 407–12.
24 Roderick PJ, Robinson AC. Life-threatening oro-pharyngeal bleeding in a haemophiliac with factor VIII inhibitors. *Clin Lab Haemat* 1988; **10**: 217–19.
25 Bogdan CJ, Strauss M, Ratnoff OD. Airway obstruction in hemophilia (factor VIII deficiency): a 28-year institutional review. *Laryngoscope* 1994; **104**: 789–94.
26 Kuhlmann TP, Abidin MR, Neal DA. Computed tomography diagnosis of acute facial swelling in an adult hemophiliac. *Ann Emerg Med* 1989; **18**: 681–3.
27 Chase CR, Hebert JC, Franham JE. Post-traumatic upper airway obstruction secondary to lingual artery hematoma. *J Trauma* 1987; **27**: 593–4.
28 Mittal R, Spero J, Lewis JH, *et al.* Patterns of gastrointestinal hemorrhage in hemophilia. *Gastroenterol* 1985; **88**: 515–22.
29 Lander E, Pechlander C, Mayr A, Mortl M, Probst A. Mallory–Weiss syndrome in a patient with hemophilia A and chronic liver disease. *Ital J Gastroenterol* 1995; **27**: 73–4.
30 Reish O, Nachum E, Naor N, Ghoshen J, Nerlob P. Hemophilia B in a neonate: unusual early spontaneous gastrointestinal bleeding. *Am J Perinatol* 1994; **11**: 192–3.
31 Lander E, Pechlaner C, Mayr A, Mortl M, Propst A. Mallory–Weiss syndrome in a patient with hemophilia A and chronic liver disease. *Ital J Gastroenterol* 1995; **27**: 73–4.
32 Singh R, Clarkston W, Zuckerman DA, Joist JH, Bacon BR. Transjugular intrahepatic portosystemic shunt for palliation of bleeding esophageal varices in a patient with severe hemophilia A, advanced HIV infection, and cirrhosis. *Am J Gastroenterol* 1993; **88**: 2112–14.
33 Griffin PH, Chopra S. Spontaneous intramural gastric hematoma: a unique presentation for hemophilia. *Am J Gastroenterol* 1985; **80**: 430–3.
34 Nigam K, Hughes RG, Murphy B. Conservative management of splenic rupture in a haemophiliac adult. *J R Coll Surg Edinb* 1998; **43**: 57.
35 Brook J, Newnam P. Spontaneous rupture of the spleen in hemophilia. *Arch Intern Med* 1965; **115**: 595–7.
36 Jones JJ, Kitchens C. Spontaneous intra-abdominal hemorrhage in hemophilia. *Arch Intern Med* 1984; **144**: 297–300.
37 Quick AJ. Emergencies in hemophilia. *Am J Med Sci* 1966; **251**: 409–16.
38 Johnson-Robbins LA, Porter JC, Horgan MJ. Splenic rupture in a newborn with hemophilia A: case report and review of the literature. *Clin Pediatr* 1999; **38**: 117–19.
39 Weintraub WH. Nonsurgical therapy of splenic rupture in a hemophiliac. *J Pediatr Surg* 1992; **27**: 1486.
40 Fort DW, Bernini JC, Johnson A, Cochran CJ, Buchanan GR. Splenic rupture in hemophilia. *Am J Pediatr Hematol Oncol* 1994; **16**: 255–9.
41 Terry NE, Boswell WC, Nonoperative management of delayed splenic rupture in a patient with hemophilia B. *J Ped Surg* 2006; **41**: 1607–9.
42 Willbanks OL, Willbanks SE. Femoral neuropathy due to retroperitoneal bleeding. *Am J Surg* 1983; **145**: 193–8.
43 McCoy HE, 3rd, Kitchens CS. Small bowel hematoma in a hemophiliac as a cause of pseudoappendicitis: diagnosis by CT imaging. *Am J Hematol* 1991; **38**: 138–9.
44 Morsman CDG, Holmes J. Traumatic hyphaema in a haemophiliac. *Br J Ophthalmology* 1990; **74**: 563.
45 Kobayashi H, Honda Y. Intraocular hemorrhage in a patient with hemophilia. *Metab Ophthalmol* 1984–5; **8**: 27–30.
46 Theobald T, Davitt BV, Shields SR. Hemorrhagic glaucoma in an infant with hemophilia, spontaneous hyphema, aniridia, and persistent iris vessels. *J AAPOS* 2001; **5**: 129–30.
47 Lifshitz T, Yermiahu T, Biedner B, Yassur Y. Traumatic total hyphema in a patient with severe hemophilia. *J Pediatr Ophthalmol Strabismus* 1986; **23**: 80–1.
48 Gilbert MS. Characterizing the hemophilic pseudotumor. *Ann NY Acad Sci* 1975; **240**: 311–15.

58 Quality of life in hemophilia

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Introduction

Hemophilia is a congenital life-threatening disorder characterized by spontaneous and post-traumatic bleeding events in joints, muscles, and other soft tissues. These manifestations, when not prevented, inexorably lead to severe pain, arthropathy, and disability with detriment of quality of life (QoL). The modern management of hemophilia has visibly influenced not only clinical symptoms, orthopedic outcome, and survival of patients, but also their perceived QoL [1].

Nevertheless, other concerns continue to challenge hemophilia management: the perceived threat of contaminating viruses or prions from replacement factor concentrates and the risk of development of inhibitory antibodies.

All these characteristics of hemophilia care require the expenditure of a huge amount of human and economic resources, in a time of increasing economic and personnel limitations [2,3]. These staggering costs must be considered in the context of not only morbidity and mortality but also QoL.

Improvement of patient's well-being and QoL has always been one of the goals of healthcare professionals and is indeed more and more regarded as one of the most relevant health outcome measures in medicine [4]. Health outcome data are essential to optimize treatments and to allocate resources in a cost-intensive chronic disease such as hemophilia where traditional outcome measures such as mortality are far less germane owing to the efficacy of therapy.

Quality of life

Several definitions, ranging from operational to more philosophical approaches, are available and well accepted in the international QoL field [5].

Definition of quality of life

A general definition of QoL was provided by the World Health Organization (WHO): "Individuals' perceptions of their position in life in the context of culture and value systems in which they live, and in relation to their goals, expectations, standards, and concerns" [6]. In medicine, the term QoL much more implies how the disease or the treatment is affecting the different aspects of life. Therefore, the term "health-related quality of life" was created: "Health-related quality of life (HR-QoL) is a multidimensional construct pertaining to the physical, emotional, mental, social and behavioral components of well-being and function as perceived by the patients and/or observers" [7]. HR-QoL is influenced not only by a disease and its treatment but also by personal characteristics such as coping or internal locus of control as well as by living conditions and socioeconomic status.

Issues concerning quality of life

Quality of life assessment is considered an important outcome measure in medicine from (i) an epidemiologic perspective describing the well-being and function of patients, (ii) a clinical perspective evaluating treatment intervention effects on well-being, and (iii) a health economics perspective, where analyses of quality and cost of care are associated.

Quality of life research is based on different theoretical models such as the concept of satisfaction, social comparison approaches, expectation models as well as the so-called needs models. A widely used utility measure is the patient preference measure assessing the importance attributed to different health status conditions. One of the most important issues in QoL assessment is the direct perception of patients. It is well known that observers overestimate some aspects of QoL of patients, whereas psychological aspects are often underestimated. It is recommended to use self-rated measures—so-called subjective measures—in which the patient is directly questioned [8]. Other rated measures, or "proxy" measures, are used in young children or patients who are unable to answer (e.g., mentally impaired patients). In this case, parents or other care-givers related with the patient should be asked.

In QoL assessment, instruments for use in adults and those for use in children are distinct. The ability to assess QoL in children with chronic conditions has increased in recent years, but it is still in need of development for the majority of pediatric health conditions. QoL assessment in children should involve the parents' perception of children's QoL as well as

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the parents' rating of their own QoL. It should also include perceptions of the siblings. These instruments for children should be especially developed considering age groups and developmental status. With small children, the parents' reports of children's well-being are necessary, while with grown-up children the comparison between children's and parents' perspectives turns out to be of interest in itself [9].

Development of quality of life instruments

For the construction of a new QoL instrument different steps must be considered: (i) development, (ii) translation (for international use), (iii) testing, and (iv) norming [10]. An instrument should be developed by specifying measurement goals by generating responses from focus groups (healthcare professionals and patients). Subsequently, items should be pretested and patients should be asked about how they understood each single item (cognitive debriefing). After item review and reduction, the questionnaire can be pilot-tested in order to examine its feasibility. In international studies the questionnaire must be translated in the respective languages following internationally recommended translation rules [11] with forward and backward translations.

Different approaches for the international scale development include (i) a sequential approach, in which the instrument is originated in one country and then translated into other languages, (ii) a parallel approach, in which the instrument is developed in parallel in different countries with common dimensions and items, and (iii) a simultaneous approach, in which the items are identified nationally and then translated into English [12].

The questionnaire must then be psychometrically tested with respect to reliability, validity, and responsiveness. Reliability is an indicator of the reproducibility of an instrument and refers to how consistently and accurately test scores measure a construct. It can be examined with the internal consistency coefficient (Cronbach's alpha), an indicator of the extent to which the items are interrelated; longitudinal consistency is usually measured with the test–retest reliability, based upon analysis of correlations between repeated measurements, or with the intraclass correlation (ICC) [13]. Validity testing examines whether an instrument measures what it intends to measure. Construct validity gives information about the theoretical relationship of the items to each other and can be examined in terms of convergent validity (comparing new items with other similar well-established questionnaires) and discriminant validity (differentiating clinical subgroups of patients). Responsiveness is the ability of a scale to detect changes over time and therefore can be tested only in longitudinal studies. Finally, the questionnaire should be normed [14] through the application of a scale to a representative sample of the national population in order to obtain information about age, gender, and others. Guidelines on the development of QoL questionnaires have been published [12,15].

Measures of quality of life

For the assessment of QoL, individual measurements, such as interviews and psychometrically constructed questionnaires, have been developed over the past 30 years. They are represented by one-dimensional measures that assess just one dimension of QoL (index, summary) and multidimensional measures that assess several dimensions of QoL. At the beginning, only generic instruments were available. These were followed by the development of disease-specific measures.

Generic instruments

Generic instruments can be used irrespective of a specific disease type and even in the general population. A short description of the most frequently used generic questionnaires is categorized by instruments for children versus instruments for adults.

Generic instruments for adults

The Medical Outcomes Study 36-Item health survey (SF-36) [16] is the most widely used generic questionnaire, translated and validated in several languages. The SF-36 consists of 36 items covering eight dimensions of general health status, namely physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, and mental health.

The Sickness Impact Profile (SIP) [17] is another measure assessing the perceived health status and consists of 136 items.

With the Nottingham Health Profile (NHP) [18] emotional, social, and physical distress is assessed. The questionnaire consists of 38 items pertaining to the dimensions covering sleep, pain, emotional reactions, social isolation, physical morbidity, and energy level.

The EuroQol (EQ-5D) [19] validated in many languages asks five questions about mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, followed by a global question concerning the actual health status, providing an utility score.

The World Health Organization Quality of Life assessment questionnaire (WHOQoL) [20] assesses respondents' perception and subjective evaluation of various aspects of life. The WHOQoL consists of six domains (physical, psychological, level of independence, social relationships, environment, spirituality/religion/personal beliefs) with overall 100 items. A short-form version with 26 items has also been developed.

Generic instruments for children

Several instruments have also been developed for the assessment of QoL in children. Most of these measures have been validated only on a national level. One of those instruments validated in different languages is the Child Health Questionnaire (CHQ) [21], which assesses QoL in children

from the parents' perspective. It consists of several subdimensions such as "general health perceptions," "physical functioning," and "mental health."

Another psychometrically tested instrument translated in several languages is the KINDL questionnaire, originally developed for the assessment of QoL in the general pediatric population. The KINDL is available as a self-administered and as a proxy version for three age groups (4–7, 8–12, and 13–16 years of age) and consists of 24 items pertaining to six dimensions (physical function, psychological well-being, self-esteem, family, friends, and school) and an additional chronic generic dimension [22].

The PedsQL has been developed in the USA [23] and has been translated into over 20 languages. The PedsQL consists of 23 items and is available as self- and parent-report forms for four age groups (2–4, 5–7, 8–12, and 13–18 years).

Disease-specific instruments

Although generic instruments allow a comparison of the investigated patient population with other patient groups or with the general population, they are not able to provide a clear pattern of symptoms or impairments related to a specific disease and are not sensitive enough to treatment consequences. For this reason, disease-specific measures are especially developed for the assessment of QoL in patients with a specific health condition, such as cancer, metabolic, or cardiovascular diseases as well as respiratory, neurologic, psychiatric, and rheumatologic conditions [24]. Only in the last decade have disease-specific QoL instruments been developed for children and adults with hemophilia in different countries [25].

Hemophilia-specific instruments for children

The first hemophilia-specific questionnaire (Haemo-QoL) [26] was developed for hemophilic children and their parents with a parallel approach in six European countries (Germany, France, Italy, Spain, the Netherlands, UK). The Haemo-QoL is available as a self-administered questionnaire for children of three age groups (4–7, 8–12, and 13–16 years of age) as well as respective proxy versions for their parents. The questionnaire was validated in 339 children from 20 hemophilia centers [27]. The psychometric structure of the questionnaire showed acceptable psychometric properties for the three age-group versions as well as the accompanying parent forms. Different versions exist for various study purposes (long versions, short versions, and index version) [28]. The Haemo-QoL has been linguistically validated up to now in 32 languages [29], which can be downloaded from the Haemo-QoL website (www.haemoqol.org); further languages are currently under development.

Another hemophilia-specific questionnaire for children is the Canadian Hemophilia Outcomes—Kids Life Assessment Tool (CHO-KLAT) [30], which was developed to include the perspectives of children with hemophilia. The CHO-KLAT is available for children of 5–18 years of age and includes parent- and self-report forms. The questionnaire consists of 35 items and has a single summary score representing overall QoL based on questions regarding treatment, physical health, family, future, feeling, understanding of haemophilia, other people and friends, and control over your life. Psychometric testing in 52 children revealed good to very good values for test–retest reliability and inter-rater reliability and showed good convergent validity, but no discriminant validity [31]. Additional languages such as Dutch, French, German, Spanish, and Mandarin are available.

The QoL questionnaire for young patients was developed in the USA and is only available as a proxy instrument for parents of hemophilic children aged 2–6 years [32]. The questionnaire consists of 39 items pertaining the following nine domains: "somatic symptoms," "physical functioning," "sleep disturbance," "stigma," "social functioning," "fear," "resentment/reaction," "energy level," "mood/behavior," and "restrictions." The questionnaire has been validated in 103 parents of hemophilic children and 249 parents of healthy controls and psychometric characteristics revealed good values for reliability in terms of internal consistency as well as moderate values for convergent and discriminant validity.

Hemophilia-specific instruments for adults

The first hemophilia-specific QoL questionnaire for adults is the Hemofilia-QoL [33], which was originally developed in Spain. It consists of 36 items assessing nine domains ("physical health," "daily activities," "joint damage," "pain," "treatment satisfaction," "treatment difficulties," "emotional functioning," "mental health," "relationships and social activity"). The Hemofilia-QoL has been validated in 121 adult patients and psychometric characteristics were acceptable in terms of reliability (internal consistency, test–retest) and validity (concurrent and discriminant) [34].

The Haem-A-QoL [25,35] was originally developed in Italy, which consists of 46 items pertaining to 10 dimensions ("physical health," "feelings," "view," "sport and leisure time," "work and school," "dealing," "treatment," "future," "family planning," "relationship") and a total score. The questionnaire has been validated in 233 adult hemophilia patients and showed quite good reliability values in terms of internal consistency and test–retest reliability as well as for validity (convergent, discriminant). The Haem-A-QoL has been linguistically validated in 32 languages [29], and further languages are currently being translated. The Haem-A-QoL has a core instrument with 27 shared items with the pediatric Haemo-QoL that allows a comparison between HR-QoL of adults and children. A specific version of the Haem-A-QoL has been developed for elderly patients.

The MedTap questionnaire (Haemo-QoL-A) was developed in the USA, Canada, Spain, and Germany [36]. The questionnaire consists of 41 items pertaining to six dimensions ("physical functioning," "role functioning," "worry," "consequences

of bleeding," "emotional impact," and "treatment concerns") and four independent items. The MedTap questionnaire was validated in 221 adult hemophilia patients and showed in the psychometric testing good to excellent values for reliability in terms of internal consistency and test–retest reliability. Validity was good for concurrent and discriminant validity [37].

The Hemolatin-QoL [38] was developed for Latin American countries (Spain, Portugal) and consists of 47 items pertaining to nine dimensions ("pain," "physical health," "emotional functioning," "social support," "activities and social functioning," "medical treatment," "mental health," "satisfaction with condition," "general well-being") and one single item for "general health." Psychometric evaluation is in progress within several Latin American countries.

Pharmacoeconomics and health-related quality of life

HR-QoL assessment is increasingly used in pharmacoeconomic evaluation. In fact, the fundamental nature of pharmacoeconomics is an estimate of costs and outcomes of alternative health programs. The outcome of a medical intervention is represented by the health status perceived by the patient and includes symptoms, performance, compliance, care satisfaction, and QoL. HR-QoL is often essential for assessing cost-effectiveness. Cost–utility analysis is a type of cost-effectiveness analysis in which effectiveness is weighted for a factor that considers HR-QoL. Cost–benefit analyses are also based on patients' preferences.

Choosing a measure—using a measure

Because of the multiplicity and diversity of instruments, physicians are often uncertain which of the existing hemophilia-specific instruments to use. The choice should be based on study-related (such as age group of patients, aim and design of the study) and instrument-related issues (such as feasibility, psychometric characteristics, aspects covered) [39]. When choosing a measure one must keep in mind the desired kind of investigation. In fact, generic instruments can measure general health status in different patient populations ("cross-illness comparison") and can allow comparison with the general population. Disease-specific measures can study specific problems of a selected cluster of patients. These measures are more sensitive than generic measures: a greater sensitivity allows detecting small changes and makes these measures suitable for the assessment of a specific treatment intervention over time ("within-subject comparison"). The choice of a specific instrument requires validation in the respective country and consideration of the reported sensitivity and even the number of items included. Moreover, the choice of a specific measure depends on the areas and dimensions mainly investigated by the instrument. In fact, health outcomes of a therapeutic intervention, namely efficacy and benefits, cannot always be predicted and/or measured, since the effects of an intervention cannot completely be foreseen.

Quality of life research in hemophilia

For an adequate assessment of HR-QoL in patients with hemophilia, validated instruments are necessary. A literature research in June 2007 detected 210 publications with the keywords "quality of life" and "hemophilia." In most of the publications QoL was only mentioned as a condition of the patient, which is considered as important. In these studies it was mainly stated that, for example, prophylactic treatment or home therapy is improving the QoL of hemophilic patients, but without measuring QoL. Literature reviews demonstrate that in those studies, where QoL assessments were included, mainly generic instruments have been used [40–42].

Results from generic instruments

One of the first QoL studies was conducted in 935 Dutch hemophilia patients and showed that hemophiliacs did not differ from the general population in the view of their own QoL [43]. By contrast, in Canada hemophilia patients showed significantly more limitations in their QoL compared with the general population [44].

In another study on clinical outcomes and resource utilization associated with hemophilia care, in more than 1000 European patients on prophylactic treatment QoL was better, assessed by the SF-36, than in patients receiving on-demand treatment [45]. Miners *et al.* [1] found in their study, in which SF-36 and EQ-5D were administered to 249 British hemophiliacs, that patients with severe hemophilia reported poorer levels of QoL. They suggested that early primary prophylaxis might increase the QoL in these patients. A French study in 116 patients with severe hemophilia showed that physical function and social relation were acceptable, whereas QoL scores in the pain dimension of the SF-36 were low [46].

Another study that administered the SF-36 to 150 Finnish patients with bleeding disorders showed that QoL levels were associated with clinical severity [47]. In a Spanish study in 70 patients using the SF-36, QoL was negatively affected by severe orthopedic impairment related to hemophilia [48], which was confirmed in Italy as well for inhibitor patients [49]. In a Canadian survey of mild, moderate, and severe hemophiliacs the Health Utility Index Mark 2 (HUI-2) and Mark 3 (HUI-3) were used. Hemophiliacs reported a greater burden of morbidity than the general population, being associated linearly with the severity of hemophilia [50]. QoL is often assessed after treatment as it was done by Schick *et al.* in 11 hemophilia patients after knee arthroplasty, using the SF-12, WOMAC, and Knee Society Score [51]. Even though several publications examined psychological issues in hemophilic patients with human immunodeficiency virus (HIV), only a few assessed QoL with appropriate instruments [52]. More impairments were found in the HRQoL domains "general

health" and "vitality" of the SF-36 in hepatitis C virus (HCV)-infected hemophilia patients [53]. Elander *et al.* found that the intensity of pain had the main influence on physical QoL assessed with the SF-36, while negative thoughts about pain had significant influence on mental quality of life [54].

HR-QoL in children (n = 27) with severe hemophilia was investigated by Liesner *et al.* in the UK in 1996 [55]. Prophylaxis significantly decreased the average number of bleeds compared with prior prophylaxis, and families reported an improved health perception. In a multicenter study concerning the effects of two prophylactic treatment regimes, 128 children with hemophilia from Sweden and the Netherlands were investigated including QoL measures [56]. Clinical scores and QoL were similar in both prophylactic groups. High-dose prophylaxis increased treatment costs significantly; however, arthropathy could be only slightly reduced after a follow-up of 17 years. In a study of 140 American children with hemophilia, Shapiro *et al.* found that those with few bleeding events had higher physical functioning scores assessed by the Child Health Questionnaire (CHQ) and were similar to the general population [57].

In an Italian case–control study, elderly hemophilia patients (≥65 years) not only showed impairments in their health status and daily activities, but also revealed significantly more emotional problems compared with controls. Hemophiliacs reported more frequently depression and had a lower HR-QoL assessed with the EQ-5D and the WHOQoL-Bref [58].

Only recently, the relationship between health economics and QoL has been examined in hemophilia patients using, for example, the standard gamble technique [59] or the quality-adjusted life years (QALYs) approach [1]. Szucs *et al.* found in their study of 50 German hemophilia patients, with regards to socioeconomic impact of treatment significant differences between them and healthy men, that the patients had greater limitations in physical activities, pain, and general health scores as assessed by the SF-36 [60]. In a huge European study in more than 1000 patients with hemophilia it was shown that prophylaxis was associated with higher costs but better QoL scores as measured by the SF-36 [3].

In an Italian study in 56 hemophilic patients combining QoL (SF-36) and utility assessment (EQ-5D), low scale values were found in the general health perceptions and higher scale values in social functioning [61]. Another Italian study (COCIS) involving 52 Italian hemophilic patients with inhibitors prospectively evaluated cost of care and QoL [2]. HR-QoL, measured with the SF-36 questionnaire, was similar to that of patients with severe hemophilia without inhibitors. In comparison to other diseases, physical functioning was similar to that of patients with diabetes and on dialysis, whereas mental well-being was comparable to that in the general population. This study showed that management of hemophilia complicated by inhibitors required high amounts of resources, but it provided a satisfactory QoL. As far as bleeding frequency in inhibitor patients is concerned, a reduced number of bleeding events owing to secondary rFVIIa prophylaxis was found to be associated with improved HRQoL across countries, as assessed by the EQ-5D [62].

In a cost–utility analysis in six hemophilic children with inhibitors, Eckert *et al.* included the Child-Health Questionnaire (CHQ) for the assessment of QoL and the EQ-5D for utility valuation [63]. HR-QoL improvements were observed in several important areas as perceived by both patients and their families and implied incremental cost per QALY.

Results from disease-specific instruments

In a Dutch study, 31 patients with severe hemophilia were investigated including a generic QoL instrument (SIP) and a disease-specific instrument, the Arthritis Impact Scale (AIMS) [64]. This latter questionnaire is an arthritis-specific measurement, but not a QoL measure in the usual sense. Physical health components of the Dutch AIMS and the SIP were significantly correlated; by contrast there were no correlations for the psychosocial component between the D-AIMS and the SIP. Another arthritis-specific questionnaire (CHAQ, Children's Health Assessment Questionnaire) was used in a Dutch study, in which hemophilic children (n = 39) on prophylactic treatment were compared with their healthy peers; no significant differences were found. Although 90% of the hemophilic children had no disabilities in activities of daily living (ADL), 79% reported that the disease impacted on their lives [65].

HR-QoL in pediatric patients with hemophilia was shown to be satisfactory: young children were only bothered in the Haemo-QoL dimension "family" and "treatment," whereas older children had higher impairments in the social dimensions, such as "perceived support" and "friends" [27]. The Haemo-QoL questionnaire also showed that the initial burden induced by prophylaxis in younger children is highly compensated by improvements in HR-QoL in older children, as indicated by impaired scores in the dimension "feeling" in smaller children and improved scores in the dimension "school and sport" in older children [66]. Using psychosocial determinants of QoL such as coping, locus of control, life satisfaction, and social support, it was apparent that QoL is dependent not only on clinical but also on psychosocial characteristics [67]. These psychosocial predictors varied across countries, although life satisfaction and social support explained the highest proportion of variance [68]. In the European ESCHQoL study significant differences in QoL were found across different regions according to factor consumption per capita; the study showed worse QoL in regions with a low factor consumption per capita (<2 IU) compared with regions with a higher factor consumption per capita (≥2 IU) [69]. In a paper by Bradley *et al.*, differences and similarities among the European Haemo-QoL and the Canadian CHO-KLAT have been described [70].

Italian adult hemophilia patients on continuous replacement therapy reported worse QoL compared with patients receiving on-demand treatment, which can be explained by

the deteriorated clinical situation of these patients [71]. Up to 64% of the variance of HRQoL in German adult patients assessed with the generic SF-36 and the hemophilia-specific Haem-A-QoL could be explained by physical performance measured with the HEP-Test-Q [72], indicating that physical activity and sports is beneficial for HRQoL [73].

Conclusion

A patient's QoL has always represented an important medical outcome together with control of pain and prolongation of survival. In hemophilia, the modern management has already been successful in improving the clinical symptoms, orthopedic status and the survival of patients. More recently, enhanced therapy and better healthcare management has also improved the more comprehensive well-being and HR-QoL of patients with hemophilia [1]. QoL assessment has become more and more popular in the field of hemophilia research in the last decade, allowing the assessment of patients' perceptions of the overall effect of hemophilia care.

The described hemophilia-specific questionnaires have been psychometrically tested and validated, some of them cross-culturally, but they still need to be implemented in international studies (observational, clinical). This is consistent with the recommendations of the Health Related Quality of Life Expert Working Group of the International Prophylaxis Study Group (IPSG). Accordingly, there is a recognized need to confirm the psychometric properties of these instruments, especially regarding specificity and responsiveness [74]. Further, differences among the measures should be investigated and characterized.

Moreover, QoL should be included in clinical trials evaluating the effects of different treatment options, or in product licensing studies or gene therapy trials. Benefits of different treatment strategies can be assessed by asking the patient how his well-being has improved because of his treatment. Based on cross-cultural QoL assessment, healthcare systems or hemophilia care resources can be compared among countries aiming to provide adequate care and to harmonize different healthcare services.

Finally, QoL questionnaires should be part of the medical armamentarium for the global assessment and care of patients with hemophilia in order to detect specific healthcare needs of individual patients. Thereby, ongoing efforts to maintain or to improve their quality of life can be enhanced [39].

References

1 Miners AH, Sabin CA, Tolley KH, Jenkinson C, Kind P, Lee CA. Assessing health-related quality-of-life in individuals with haemophilia. *Haemophilia* 1999; **5**: 378–85.

2 Gringeri A, Mantovani LG, Scalone L, Mannucci PM. Cost of care and quality of life in hemophilia complicated by inhibitors: the COCIS Study Group. *Blood* 2003; **102**: 2358–63.

3 Schramm W, Royal S, Kroner B, Berntorp E, Giangrande P, Ludlam C, Gringeri A, Berger K, Szucs T; for the European haemophilia economic study group. Clinical outcomes and resource utilization associated with haemophilia care in Europe. *Haemophilia* 2002; **8**: 33–43.

4 Cella DF, Tulsky DS. Measuring quality of life today: methodological aspects. *Oncology* 1990; **4**: 29–38.

5 Spilker B. *Quality of Life Assessment in Clinical Trials*. New York: Raven Press, 1996.

6 Orley J and the WHOQOL-Group. The Development of the WHO Quality of Life assessment instruments (The WHOQOL). In: Orley J, Kuyken W (eds.) *Quality of Life Assessment. International Perspectives*. Berlin: Springer Verlag, 1994: 41–57.

7 Bullinger M. Quality of life-definition, conceptualization and implications—a methodologist's view. *Thero Surg* 1991; **6**: 143–9.

8 Bowling A. *Measuring Health. A Review of Quality of Life Measurement Scales. Milton Keynes*. Philadelphia: Open University Press, 1991.

9 Eiser C, Morse R. A review of measure of quality of life for children with chronic illness. *Arch Dis Child* 2001; **84**: 205–11.

10 Juniper E, Guyatt GH, Jaeschke R. How to develop and validate a new health-related quality of life instrument. In: Spilker B (ed.) *Quality of Life and Pharmaeconomics in Clinical Trials*. Philadelphia: Lippincott-Raven, 1996: 49–56.

11 Acquadro C, Jambon B, Ellis D, Marquis P. Language and translation issues. In: Spilker B (ed.) *Quality of Life and Pharmaeconomics in Clinical Trials*. Philadelphia: Lippincott-Raven, 1996: 575–85.

12 Bullinger M, Anderson R, Cella D, Aaronson N. Developing and evaluating cross-cultural instruments from minimum requirements to optimal models. *Qual Life Res* 1993; **2**: 451–9.

13 Guyatt G, Kirshner B, Jaeschke R. Measuring health status: what are the necessary measurement properties? *J Clin Epidemiol* 1992; **45**: 1341–5.

14 Guyatt GH, Feeny DH, Patrick DL. Measuring health-related quality of life. *Ann Intern Med* 1993; **118**: 622–9.

15 Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. *Spine* 2000; **25**: 3186–91.

16 Ware JE, Snow KK, Kosiniski M, Gandek B. *SF-36 Health Survey Manual and Interpretation Guide*. Boston: New England Medical Centre, 1993.

17 Bergner M, Bobbit RA, Carter WB, Gilson BS. The Sickness Impact Profile: development and final revision of a health status measure. *Med Care* 1981; **19**: 787–805.

18 Hunt SM, McKenna SP, McEwen J, Williams J, Papp E. The Nottingham Health Profile: subjective health status and medical consultations. *Soc Sci Med* 1981; **15A**: 221–9.

19 Kind P. The EuroQol Instrument: an index of health-related quality of life. In: Spilker B (ed.) *Quality of Life and Pharmaeconomics in Clinical Trials*. Philadelphia: Lippincott-Raven, 1996: 191–201.

20 Power M, Harper A, Bullinger M. The World Health Organization WHOQOL-100: tests of the universality of Quality of Life in 15 different cultural groups worldwide. *Health Psychol* 1999; **18**: 495–505.

21 Landgraf I, Abetz L, Ware JE. *Child Health Questionnaire (CHQ): A user's manual*. Boston: The Health Institute Press, 1997.

22 Ravens-Sieberer V, Mullinger M. Assessing the health related qualità of life in chronically ill children with the German KINDL. First psychometric and content-analytic results. *QoL Research* 1999; **7**: 399–408.

23 Varni JW, Seid M, Rode CA. The PedsQL: measurement model for the pediatric quality of life inventory. *Med Care* 1999; **37**: 126–39.

24 Bowling A. *Measuring Disease. A Review of Disease-specific Quality of Life Measurement Scales*. Buckingham: Open University Press, 2001.

25 von Mackensen S, Gringeri A. Quality of life in haemophilia. In: Preedy VR, Watson RR (eds.) *Handbook of Disease Burdens and Quality of Life Measures*. Heidelberg: Springer, 2009; Vol 3: 1895–1920.

26 Bullinger M, v. Mackensen S, Fischer K, *et al.* Pilot testing of the Haemo-QoL quality of life questionnaire for haemophiliac children in six European countries. *Haemophilia* 2002; **8**(Suppl. 2): 47–54.

27 v. Mackensen S, Bullinger M, and the Haemo-QoL Group. Development and testing of an instrument to assess the quality of life of children with haemophilia in Europe (Haemo-QoL). *Haemophilia* 2004; **10**(Suppl. 1): 17–25.

28 Pollak E, Mühlan H, v. Mackensen S, Bullinger M, and the Haemo-QoL Group. The Haemo-QoL Index: developing a short measure for health-related quality of life assessment in children and adolescents with haemophilia. *Haemophilia* 2006; **12**: 384–92.

29 Chevallet L, Weatherall J, von Mackensen S. Linguistic validation of the Haemo-QoL and Haem-A-QoL for use in international studies. *Value in Health* 2008; **11**: PSY44: A165.

30 Young N, Bradley C, Blanchette V, Wakefield C, Barnard D, McCusker P. Development of a health-related quality of life measure for boys with hemophilia: The Canadian Hemophilia Outcomes-Kids Life Assessment Tool (CHO-KLAT). *Haemophilia* 2004; **10**: 34–43.

31 Young NL, Bradley CS, Wakefield CD, Barnard D, Blanchette VS, McCusker PJ. How well does the Canadian Haemophilia Outcomes-Kids' Life Assessment Tool (CHO-KLAT) measure the quality of life of boys with haemophilia? *Pediatr Blood Cancer* 2006; **47**: 305–11.

32 Manco-Johnson M, Morrissey-Harding G, Edelman-Lewis B, Oster G, Larson P. Development and validation of a measure of disease-specific quality of life in young children with haemophilia. *Haemophilia* 2004; **10**: 34–41.

33 Arranz P, Remor E, Quintana M, *et al.*, and Hemofilia-QoL Group. Development of a new disease-specific quality-of-life questionnaire to adults living with haemophilia. *Haemophilia* 2004; **10**: 376–82.

34 Remor E, Arranz P, Quintana M, *et al.*, in representation of the Hemofilia-QoL Project Group. Psychometric field study of the new haemophilia quality of life questionnaire for adults: The Hemofilia-QoL. *Haemophilia* 2005; **11**: 603–10.

35 von Mackensen S, Gringeri A, Santoni L, *et al.* Development and pilot testing of a disease-specific quality of life questionnaire for adult patients with haemophilia (Haem-A-QoL). *Blood* 2004; **104**: 2214 (Abstract).

36 Flood E, Szende A, Rentz A, *et al.* Health-related quality of life in adults with hemophilia: the patient perspective. *J Thromb Haemost* 2003; **1**(Suppl. 1): P0657 (Abstract).

37 Rentz A, Flood E, Altisent C, *et al.* Cross-cultural development and psychometric evaluation of a patient-reported health-related quality of life questionnaire for adults with haemophilia. *Haemophilia* 2008; **14**: 1023–34.

38 Remor E, and the Hemolatin-QoL Group. Hemolatin-QoL: Desarrollo de una medida específica para la evaluación de la calidad de vida en pacientes adultos con hemofilia en America-Latina: el Hemolatin-QoL. *Revista Interamericana de Psicologia* 2005; **39**: 211–20.

39 Gringeri A, v. Mackensen S. Quality of life in haemophilia. *Haemophilia* 2008; **14**(Suppl. 3): 19–25.

40 Fischer K, van der Bom JG, van den Berg HM. Health-related quality of life as outcome parameter in haemophilia treatment. *Haemophilia* 2003; **9**(Suppl. 1): 75–81.

41 Szende A, Schramm W, Flood E, Larson P, Gorina E, Rentz AM, Snyder L. Health-related quality of life assessment in adult haemophilia patients: a systematic review and evaluation of instruments. *Haemophilia* 2003; **9**: 678–87.

42 Remor E, Young NL, von Mackensen S, Lopatina EG. Disease-specific quality-of-life measurement tools for haemophilia patients. *Haemophilia* 2004; **10**(Suppl. 4): 30–4.

43 Rosendaal FR, Smit C, Varekamp I, *et al.* Modern hemophilia treatment: medical improvements and quality of life. *J Intern Med* 1990; **228**: 633–40.

44 Walsh M, Macgregor D, Stuckless S, Barrett B, Kawaja M, Scully MF. Health-related quality of life in a cohort of adult patients with mild hemophilia A. *J Thromb Haemost* 2008; **6**: 755–61.

45 Royal S, Schramm W, Berntorp E, *et al.* Quality-of-life differences between prophylactic and on-demand factor replacement therapy in European haemophilia patients. *Haemophilia* 2002; **8**: 44–50.

46 Molho P, Rolland N, Lebrun T, *et al.* Epidemiological survey of the orthopaedic status of severe haemophilia A and B patients in France. The French Study Group. *Haemophilia* 2000; **6**: 23–32.

47 Solovieva S. Clinical severity of disease, functional disability and health-related quality of life. Three-year follow-up study of 150 Finnish patients with coagulation disorders. *Haemophilia* 2001; **7**: 53–63.

48 Aznar J, Magall M, Querol F, Gorina E, Tusell J. The orthopaedic status of severe haemophiliacs in Spain. *Haemophilia* 2000; **6**: 170–6.

49 Scalone L, Mantovani LG, Mannucci PM, Gringeri A; The COCIS Study Investigators. Quality of life is associated to the orthopaedic status in haemophilic patients with inhibitors. *Haemophilia* 2006; **12**: 154–62.

50 Barr RD, Saleh M, Furlong W, *et al.* Health status and health-related quality of life associated with hemophilia. *Am J Hematol* 2002; **71**: 152–60.

51 Schick M, Stucki G, Rodriguez M, *et al.* Haemophilic: arthropathy: assessment of quality of life after total knee arthroplasty. *Clin Rheumatol* 1999; **18**: 468–72.

52 Bussing R, Johnson S. Psychosocial issues in hemophilia before and after the HIV crisis: a review of current research. *Gen Hosp Psychiatry* 1992; **14**: 387–403.

53 Posthouwer D, Plug I, van der Bom JG, Fischer K, Rosendaal FR, Mauser-Bunschoten EP. Hepatitis C and health-related quality of life among patients with hemophilia. *Haematologica* 2005; **90**: 846–50.

54 Elander J, Robinson G, Mitchell K, Morris J. An assessment of the relative influence of pain coping, negative thoughts about pain, and pain acceptance on health-related quality of life among people with hemophilia. *Pain* 2009; **145**: 169–75.

55 Liesner RJ, Khair K, Hann IM. The impact of prophylactic treatment on children with severe haemophilia. *Br J Haematol* 1996; **92**: 973–8.

56 Fischer K, Astermark J, Van-Der-Bom J, *et al.* Prophylactic treatment for severe haemophilia: Comparison of an intermediate-dose to a high dose regimen. *Haemophilia* 2002; **8**: 753–60.

57 Shapiro A, Donfield S, Lynn H, *et al.* Defining the impact of hemophilia: the Academic Achievement in Children with Hemophilia Study. *Pediatrics* 2001; 108: E105.

58 Siboni SM, Mannucci PM, Gringeri A, *et al.*, for the Italian Association of Haemophilia Centres (AICE). Health status and

quality of life in elderly persons with severe hemophilia born before the advent of regular replacement therapy: a case–control study. *J Thromb Haemost* 2009; **7**: 780–6.
59 Naraine V, Risebrough N, Oh P, *et al.* Health-related quality-of-life treatments for severe hemophilia: Utility measurements using the Standard Gamble technique. *Haemophilia* 2002; **8**: 112–20.
60 Szucs TD, Offner A & Schramm W. Socioeconomic impact of hemophilia care. Results of a pilot study. *Haemophilia* 1996; **2**: 211–17.
61 Trippoli S, Vaiani M, Linari S, Longo G, Morfini M, Messori A. Multivariate analysis of factors influencing quality of life and utility in patients with haemophilia. *Haematologica* 2001; **86**: 722–8.
62 Hoots WK, Ebbesen LS, Konkle BA, *et al.*; Novoseven (F7HAEM-1505) Investigators. Secondary prophylaxis with recombinant activated factor VII improves health-related quality of life of haemophilia patients with inhibitors. *Haemophilia* 2008; **14**: 466–75.
63 Ekert H, Brewin T, Boey W, Davey P, Tilden D. Cost-utility analysis of recombinant factor VIIa (NovaSeven) in six children with long-standing inhibitors to factor VIII or IX. *Haemophilia* 2001; **7**: 279–85.
64 de-Joode E, van-Meeteren N, van-den-Berg H, de-Kleijn P, Helders P. Validity of health status measurement with the Dutch Arthritis Impact Measurement Scale 2 in individuals with severe haemophilia. *Haemophilia* 2001; **7**: 190–7.
65 Schoenmakers M, Gulmans V, Helders P, van-der-Berg H. Motor performance and disability in Dutch children with haemophilia: a comparison with their healthy peers. *Haemophilia* 2001; **7**: 293–8.
66 Gringeri A, v. Mackensen S, Auerswald G, *et al.*, for the Haemo-QoL Study*. Health status and health-related quality of life of children with haemophilia from six west European countries. *Haemophilia* 2004; **10**(Suppl. 1): 26–33.
67 Bullinger M, v. Mackensen S, the Haemo-QoL Group. Quality of life in children and families with bleeding disorders. *J Pediatr Haematol Oncol* 2003; **25**: 64–7.
68 Bullinger M, von Mackensen S. Psycho-social determinants of quality of life in children and adolescents with haemophilia—a cross-cultural approach. *Clin Psychol Psychother* 2008; **15**: 164–72.
69 Bullinger M, Gringeri A, von Mackensen S. Quality of life of young patients with haemophilia in Europe. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 2008; **51**: 637–45.
70 Bradley CS, Bullinger M, McCusker PJ, Wakefield CD, Blanchette VS, Young NL. Comparing two measures of quality of life for children with haemophilia: the CHO-KLAT and the Haemo-QoL. *Haemophilia* 2006; **12**: 643–53.
71 von Mackensen S, Scalone L, Ravera S, Mantovani L, Gringeri A, for the COCHE Study Group. Assessment of health-related quality of life in patients with haemophilia with the newly developed haemophilia-specific instrument (Haem-A-QoL). *Value Health* 2005; **8**: A127 (Abstract PHM5).
72 von Mackensen S, Czepa D, Herbsleb M, Ziezo R, Hilberg T. Quality of life and subjective physical performance in adult haemophilia patients. *Haemostaseologie* 2008; **28**(Suppl. 1): S118–S119.
73 von Mackensen S. Quality of life and sports activities in patients with haemophilia. *Haemophilia* 2007; **13**(Suppl. 2): 38–43.
74 Bullinger M, Globe D, Wasserman J, Young NL, von Mackensen S, on behalf of the Health Related Quality of Life Expert Working Group of the International Prophylaxis Study Group (IPSG). Challenges of patient-reported outcome assessment in haemophilia care—a state of the art review. *Value Health* 2009; Mar 11. [Epub ahead of print]

59 The economics of hemophilia treatment

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Introduction

Health economics is the application of economic theory and method in the analysis of questions concerning people's health. There are two fundamental conditions in all economic analyses. The first is that resources in terms of labor (human capital) and equipment, energy, and raw materials (capital) are scarce with respect to people's needs. The second is that resources can be used in alternative ways. Together, these two conditions describe the starting point not only for all economic analyses regarding health but also for analyses of the labor market, for financial markets or for international trade. The common starting point also means that there is not a separate theory of the individual as a consumer in the market, another for the individual as an employee, and a third theory to describe the individual as a patient in health care. To be able to apply the same theory in different settings, the theory needs to rest on general assumptions. The strength of a general theory is that the specific results may easily be added to previous results and thus understood in a broader perspective.

Using the economic formulation of choice problems on resources within health care does not contradict the medical decision problem or the ethical considerations, but should be seen as complementary information. The economic analysis contributes with a systematic comparison of cost of resources and consequences of resource use. Table 59.1 lists key concepts for health economic analyses.

Health economic methods and the economic perspective

The health economic problem may be formulated in terms of the choice between relevant alternatives with the purpose of obtaining as much health and welfare as possible with respect to resources used. Health economic questions, then, concern individual health-related behavior, the healthcare system as a system for production, insurance against healthcare cost and loss of income as a consequence of illness or injury, distributional and equity aspects, as well as the consequences of globalization on health.

Health economic research, as all other economic research, applies an individualistic perspective. The starting point is that the sum of all single actors' (individuals, companies, organizations, authorities, etc.) reactions on events, and political actions that will decide the consequences for both the single actor and for society as a whole. For example, the primary interest of economic analyses is *not* to find out the reaction of the single individual on more information on the negative consequences of tobacco smoking, on a tax increase on cigarettes, or on prohibition of smoking in public places. The primary research question concerns the *joint effect* on public health, on the economic situation of families, and on the expenditure of the social insurance system.

In economic theory, abstract models are used to describe the main feature of the situation to be analyzed and the assumptions that make it possible to study the phenomena or behaviors of interest. One basic assumption for economic models is that agents (individuals as patients or professionals, firms, organizations, etc.) are rational. By rationality, economists mean that the agent acts as if he or she strives toward an apparent goal with the purpose of reaching this goal to the greatest possible extent. The economic perspective has a strict individualistic basis from which follows that it is not possible to determine what would be rational for someone else. Nevertheless, some types of behavior may seem irrational—someone who smokes too much or exercises too little, for example, given the amount of knowledge regarding the impact of lifestyle factors on health. Economic models are not conditioned on all individuals (or even one) striving toward maximum health. The basic premise for health economists is that other things in life may generate meaning and pleasure and that individuals therefore make trade-offs between health and other desirable goals. The goals will, of course, vary from individual to individual. For example, the behaviors and values of the peer groups are important for teenagers and smokers derive pleasure from the cigarette while smoking. For single individuals, economists usually assume that the goal is

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Table 59.1 Key health economic concepts in alphabetical order.

Term	Description
Cost	The value of resource use. *See also* opportunity cost
Cost-efficiency	Describes cost of a treatment strategy in relation to its effect/outcome
Direct costs	The value of total resource use within the healthcare system and in other sectors because of illness or injury. Restricted to costs based on resource use within outpatient and inpatient care and of pharmaceuticals in studies applying a healthcare sector perspective. Included in societal costs
Dominant strategy	Term in economic evaluations. A treatment alternative that renders better outcomes and has lower cost than the comparisons, or that is equally effective but costs less
Incentive	Motivation. Factors that describe the conditions under which individuals and other agents acts. May be financial, e.g., remuneration to doctors, nurses, or hospital departments for produced care or the price a patient pays for a healthcare visit
Indirect cost	The value of lost production as a consequence of an illness or injury that may concern the ill person her-/himself but also family and friends. In practice, the valuation of lost production is often restricted to the person with the illness or injury. Included in societal costs
Incremental cost-efficiency ratio (ICER)	Term within health economic evaluations. Denotes the ratio of the difference in cost to the difference in effect for the two alternatives under comparison. See Equation 1
Intangible cost	Cost in terms of reduced quality of life as a consequence of illness
Opportunity cost	A principle to value resource use building on the assumption that all resources have (at least) one alternative use. Choosing one alternative implies forgoing the value that could be obtained with an alternative use. The opportunity cost measures the highest valued alternative use of the resource. The identification of the opportunity cost depends on the decision situation
Quality-adjusted life-year (QALY)	An index measure that weighs changes in life-years gained (quantity) by the morbidity (quality) of life in those years
Societal cost	The sum of direct, indirect, and intangible cost in society as a result of an illness or injury

to obtain as much utility or well-being as possible in life. The firm/corporation is assumed to strive toward as much profit as possible, and a hospital toward low cost for the health care provided to the general public, politicians toward as many votes from their constituency as possible, and public authorities toward a bigger organization.

Agents are, however, restricted in their actions and cannot usually reach all goals. For the hospital, this means that if the needs of the general public shall be satisfied, costs may not be reduced beyond certain limits. A firm must consider the conditions for production, wages, and other production cost as well as the price at which the goods or services may sell. The individual is constrained by time (24 hours per day) and by his or her income and other economic resources. There may also be rules that constrain the agent's actions, e.g. the doctor's prescription to obtain certain drugs.

The goal and questions in health economic analyses cover a vast area. However, the most common economic studies within the medical field represent comparative evaluations of alternative treatment strategies and descriptive studies of costs of illness.

Health economic analyses in practice

The past decade has seen an increasing number of authorities and government bodies within the healthcare arena in Europe and North America where health economic evaluations are an integrated part in the decision-making process. The National Institute for Clinical Excellence (NICE; www.nice.org.uk) was established in the UK in 1999 with an aim to decide upon what treatments should be used within the National Health Service based on scientific evidence of efficacy and effectiveness and on the cost-efficiency of the treatment. The Dental and Pharmaceutical Benefits Agency TLV (www.tlv.se) was established in Sweden in 2002 with the mandate to decide whether a pharmaceutical product should be subsidized based on its cost-efficiency. In practice, the TLV influences the Swedish healthcare market since drugs that cannot provide evidence on cost-efficiency at the price demanded by the producer will not be subsidized. Nonsubsidized drugs may still be sold on the Swedish market provided they have received approval by the Medical Products Agency, but the lack of public subsidy will, in practice, often imply that the drug is withdrawn from the market. Other examples of countries with government bodies using input from health economic evaluations include Canada, Scotland, and Denmark.

Health economic evaluation

A health economic evaluation (sometimes termed comparative effectiveness research) is a systematic comparison of costs and consequences for two or more relevant treatment alternatives. In hemophilia, examples of such treatment alternatives include plasma-derived versus recombinant factor concentrates in the treatment of hemophilia patients, immediate surgery compared with watchful waiting for the patient with arthrodeses, and individual patient education programs versus education in groups. A minimum for an economic evaluation is two

alternatives, but more than two alternatives may be relevant for the clinical decision problem and then pair-wise comparisons will be performed. For the health economic evaluation it is necessary to combine health economic and clinical expertise in the formulation of the relevant decision problem and the characterization each of the treatment alternatives.

Perspective

Health economic evaluations may be conducted using different perspectives. If the analysis concerns the allocation of resources within a given healthcare budget, the evaluation may take a healthcare sector perspective. The comparison of costs and consequences will then include direct costs that accrue within the healthcare sector and outcome measures of relevance (e.g., reduced number of hospitalizations or bleeds or patient-centered measures such as health-related quality of life). Costs in an evaluation from the health sector perspective will then include all costs at the hemophilia clinic: These may include costs of contacts with, for example, doctors, nurses, and physiotherapists; the hospital days because of hemophilia; and the treatments provided because the patient has hemophilia, including factor concentrates and other pharmaceuticals and infusion aids. Resource use and the cost that falls on other sectors are not included.

The limitation of the health sector perspective is that it ignores costs and consequences in other sectors impacted by decisions in the healthcare sector. Examples may include early discharge from hospital requiring a family member to be absent from work and taking care of the patient instead, or a more ambitious annual control program requiring patients to come to the hospital on two days instead of one day.

Analyses from the societal perspective consider costs and consequences of the treatment alternative that may occur within the health sector and within other sectors of society. With the broader societal perspective, questions on whether it is worthwhile to expand the current healthcare budget when competing alternatives may, for instance, be more money to schools or to building roads. It follows that this perspective is also the most demanding in terms of obtaining valid data and of understanding the full range of costs and consequences of decisions. Analyses in hemophilia care from the societal perspective should then include *inter alia* consequences on the labor market of alternative treatment strategies in terms of sick leave, unemployment, and early retirement.

Three methods for economic evaluations

There are three alternative tools for systematic analyses of costs and consequences for the comparison of healthcare programs:

- cost-effectiveness analysis (CEA);
- cost–utility analysis (CUA); and
- cost–benefit analysis (CBA).

Table 59.2 summarizes the information needed for each type of analysis and how results are interpreted, and may be applied for each of the three methods.

A health economic evaluation is a comparison of (at least) two treatment alternatives. The result from cost-effectiveness and cost–utility analyses are presented as a ratio of pair-wise comparisons of differences in cost to differences in effect. Equation 1 presents the incremental cost-effectiveness ratio (ICER) schematically for two alternative treatments, A and B.

$$ICER = \frac{Cost_{Treatment\ A} - Cost_{Treatment\ B}}{Effect_{Treatment\ A} - Effect_{Treatment\ B}} \quad (1)$$

where $cost_A$ and $cost_B$ are the cost of treatments A and B, respectively, and $effect_A$ and $effect_B$ are the effects associated

Table 59.2 Characteristics of three alternative methods for full economic evaluation.

Method	Cost	Consequence	Recommendation for decision-making	Tool for priority setting
Cost-effectiveness analysis (CEA)	Resource use measured in monetary terms (euro, dollar, pound, etc.)	Single-dimension consequences measured in physical units. For example, life-years gained, reduction in number of bleeds per year, or reduction in pain from start of bleed	Choose the alternative with the lowest cost per unit gained	Enables comparisons between treatment alternatives with the same relevant outcome dimensions
Cost–utility analysis (CUA)	Resource use measured in monetary terms (euro, dollar, pound, etc.)	Consequences measured in quality-adjusted life-years (QALYs), which simultaneously measure reduced morbidity (quality gains) and reduced mortality (quantity gains)	Choose the alternative with the lowest cost per QALY gained	Enables comparisons between treatment alternatives within different parts of the healthcare sector using common outcome
Cost–benefit analysis (CBA)	Resource use measured in monetary terms (euro, dollar, pound, etc.)	Consequences measured in monetary terms (euro, dollar, pound, etc.)	Choose the alternative with the biggest net benefit (sum of benefits – sum of costs)	Enables comparisons between alternatives in different sectors of society

with treatments A and B, respectively. In a CEA, effects are measured in natural units, for instance life-years gained or bleeding episodes avoided. From the hemophilia literature, Miners and colleagues reported some 10 years ago on the results from a CEA of switching from on-demand to prophylaxis using averted bleeds as outcome measure [1]. Bleeding episodes avoided is a relevant outcome measure within hemophilia care, but it is disease specific and cannot be meaningfully used in comparisons with treatments in other hospital departments. Life-years gained may be applicable for many diseases with risk of fatality, but it may not be sensitive in catching differences in quality of life.

A special case of cost-effectiveness would be when two alternative treatments under consideration achieve the same outcome to the same extent, e.g., pharmaceuticals containing the same active substances but sold under different brand names. When outcome is equal, the decision is seemingly trivial and a comparison of costs of the alternatives would then suffice. Such a cost-minimization analysis is considered as a partial evaluation since it examines only costs in practice.

For general comparisons, the analyst needs outcome measures that are not tailored for disease-specific groups. Health economists have developed an index to measure health-related quality of life denoted quality-adjusted life-years (QALYs). Over the last decade, QALYs have become a well-established tool for health economic evaluations used by authorities such as NICE (UK) and TLV (Sweden).

Cost–utility analyses relate costs to QALYs gained. The measure of QALYs is either based on the patient's own valuation of quality of life in his or her present state or imputed from valuations of specified health states made by members of the general public. For the purpose of evaluation it is important that the instrument used to value the health state is based on people's preferences. The methods of measurement are based on how people choose between alternative health states. Drummond and coauthors provide a well-written introductory textbook on methods for such evaluations [2].

A CUA using decision-analytic modeling compared Canadian tailored prophylaxis, primary prophylaxis, and on-demand therapy in young children with severe hemophilia using longitudinal Canadian data and estimates from the published literature [3]. The authors concluded that prophylaxis considerably improved clinical outcomes and quality of life compared with on-demand treatment, but with a substantial cost.

Cost-effectiveness analysis and CUA share methodological features. Both methods present results as a ratio where the cost is related to the chosen outcome measure. The incremental cost-effectiveness ratios (ICER; Equation 1) may then be used to rank alternatives within a given healthcare budget.

The third method for economic evaluation, the CBA, measures both cost and consequences in monetary units (euro, dollar, pound, etc.). Using a common metric, results are expressed as net benefits and a treatment alternative, where sum of benefits exceed the sum of costs, is then worthwhile. The CBA then enables comparisons of resource use in different sectors.

The methods applied by health economists to estimate monetary values of consequences of health programs are similar to the methods used within environmental economics (see Bateman and colleagues for a comprehensive overview [4]). The methods have developed techniques to elicit people's willingness to pay for nonmarket goods and services, hence the concept of willingness to pay (WTP). Several features have been developed to handle the fact that respondents do not normally face a situation where they are actually paying the full cost of healthcare treatment at the point of treatment but are covered through various sorts of private or social insurance.

Two examples of studies in hemophilia are Steen Carlsson and collaborators, who estimated the average WTP for on-demand and prophylactic treatment using a random sample from the general population, and Eastaugh, who estimated the WTP for treatment of von Willebrand disease in a convenience sample of patients with the disease [5,6]. Steen Carlsson and collaborators also compared the estimated willingness to pay to the average cost per tax payer estimated within the project [7] and found that although both on-demand and prophylactic treatment had positive estimated net benefits, the net benefits were higher for prophylaxis.

Study design

The appropriateness of design of an evaluation by CEA, CUA, or CBA depends on the characteristics of the economic problem and on data availability. Economic evaluations may rest on patient-level data, either observational data collected directly for the purpose of the evaluation or, more commonly, piggy-backed on a clinical trial. Another strategy when no trial or observational data are at hand is to use decision-analytic modeling. Decision-analytic modeling provides a framework for decision-making under uncertainty when data may only be available from smaller case studies and from expert opinion, or when data from several published sources need to be combined to address the economic problem at hand.

For example, health economic analyses of alternative treatment strategies for hemophilia patients who have developed inhibitors to factor VIII concentrate may serve as an illustration of choice of design strategies. Until recently, there were no larger-scale trials on inhibitor treatment from which economic data had been retrieved. Three publications used decision-analytic modeling to compare the two alternative hemostatic agents used in the treatment of patients with inhibitors: activated prothrombin complex concentrate (aPCC) and recombinant factor VIIa (rFVIIa) [8–10]. The comparison concerned treatment with the two alternative bypassing agents during one bleeding episode, but the conclusions were ambiguous.

Scientific journals demand that the assumptions and the structure of the model are clearly reported, enabling re-estimations using other data on costs and effects or changing

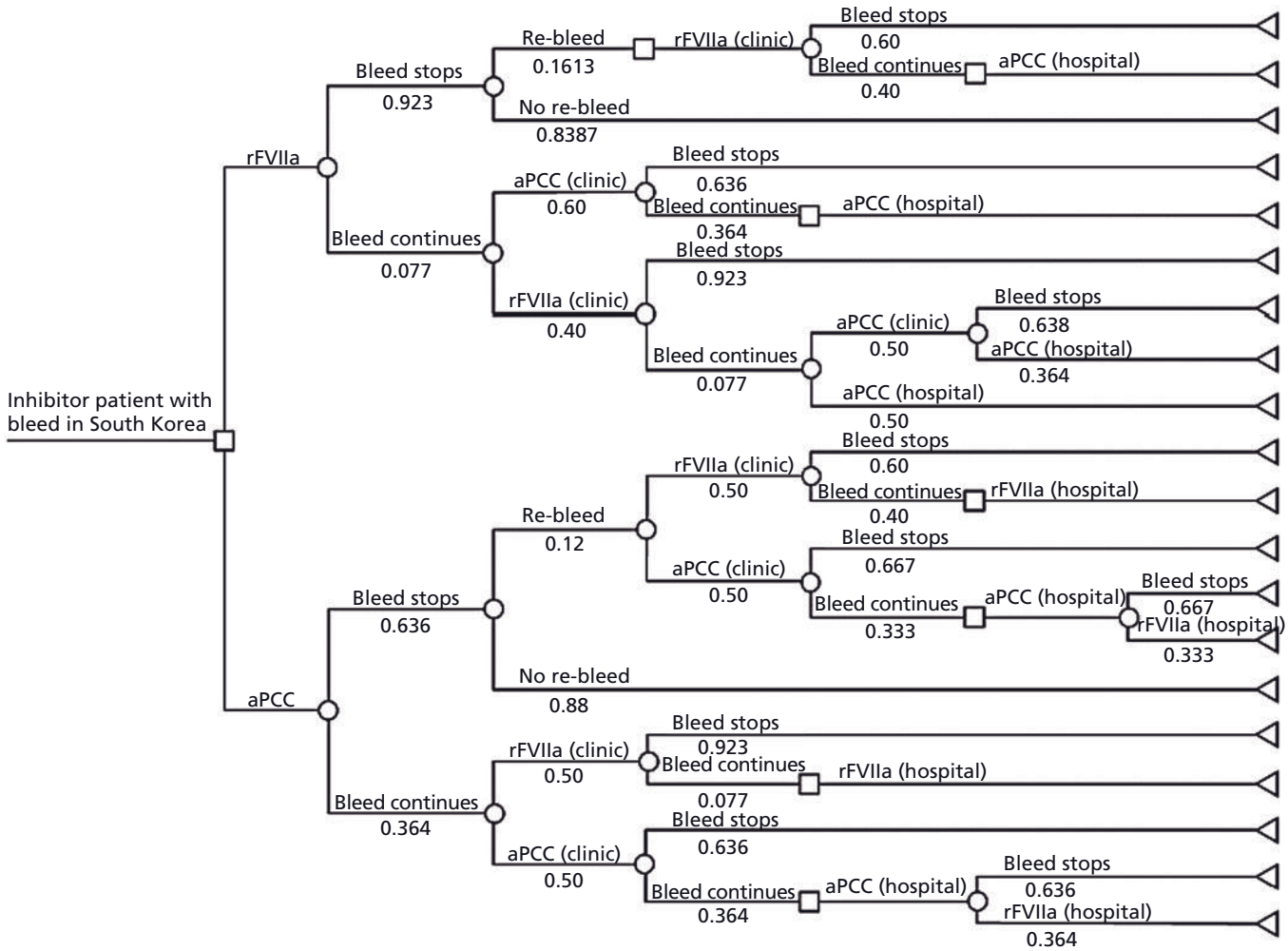

Figure 59.1 Decision model structure. Reproduced from [13] with permission.

the assumptions to fit other clinical settings. Figure 59.1 shows an example of the decision tree visualizing the model structure used by You and collaborators for analyzing the cost-effectiveness of bypassing agents. The first head-to-head trial on the two alternative bypassing agents, the FENOC study, was published by Astermark and collaborators [11]. The clinical data were also used for a piggy-back cost-effectiveness analysis where patient-rated outcomes were used for measuring effect: treatment effective, bleeding stopped, and pain reduction [12], and the results reported as ICERs (see also Equation 1) for aPCC compared with rFVIIa. The analysis also showed the role of prices on resource use for the cost-effectiveness results and the ICER.

The research design based on observational data has the advantage that it may capture the variation of actual patients and in the daily clinical work. However, comparisons between alternative treatment strategies may not be feasible based on observational data where differences in treatment will depend on differences in patient characteristics and need for treatment. On the other hand, protocol-based trial data may be well suited for comparisons when patients are randomized to the alternative treatment strategies. The drawback is that the trial situation may entail strict inclusion and exclusion criteria and may have study-driven compliance limiting the generalizability on a large scale. Decision-analytic modeling is attractive since it enables sensitivity analysis and easy adaptation when new results on efficacy, efficiency, and cost are available, but is sensitive to assumptions. In conclusion, the alternative designs all have their place in supplying decision-makers with information, and sometimes the combination of studies provide the clear picture.

Assessment of the quality of economic evaluations

Health economic analyses are increasingly demanded by authorities and by commercial organizations and enterprises in order to inform decision-making in the health system. To meet the need for assessing the quality of a health economic evaluation, checklists have been developed. One example of a checklist—known as Drummonds' 10 points and covering

issues including well-defined and answerable question, comprehensive description of the competing alternatives and inclusion of all relevant alternatives, the accurate measurement of costs and effects and the use of adjustment for differentials in timing and sensitivity analyses—has been published by Drummond and collaborators [2] and may also be found in an adapted version on the homepage of the *British Medical Journal* (http://resources.bmj.com/bmj/authors/checklists-forms/health-economics).

Conclusion

The starting point for all economic analyses is that resources are scarce with respect to people's needs and that resources will always have alternative uses. The scarcity of resources may be especially evident in healthcare, and therefore tools to make systematic considerations of the costs and consequences of the available alternatives are an important input in decision-making. This chapter gave an overview the economic scientific perspective and of three methods for economic evaluations of healthcare programs—cost-effectiveness, cost–utility, and cost–benefit analyses.

References

1 Miners A, Sabin C, Tolley K, Lee C. Assessing the effectiveness and cost-effectiveness of prophylaxis against bleeding in patients with severe haemophilia and severe von Willebrand's disease. *J Intern Med* 1998; **244**: 515–22.

2 Drummond M, Sculpher M, Torrance G, O'Brien B, Stoddart G. *Methods for the Economic Evaluation of Health Care Programmes*, 3rd edn. Oxford: Oxford University Press, 2005.

3 Risebrough N, Oh P, Blanchette V, Curtin J, Hitzler J, Feldman B. Cost–utility analysis of Canadian tailored prophylaxis, primary prophylaxis and on-demand therapy in young children with severe haemophilia A. *Haemophilia* 2008; **14**: 743–52.

4 Bateman I, Carson R, Day B, *et al*. *Economic valuation with stated preference techniques: a manual*. Cheltenham, UK: Edward Elgar, 2002.

5 Eastaugh SR. Willingness to pay in treatment of bleeding disorders. *Int J Technol Assess Health Care* 2000; **16**: 706–10.

6 Steen Carlsson K, Höjgård S, Lethagen S, Lindgren A, Berntorp E, Lindgren B. Willingness to pay for on-demand and prophylactic treatment for severe haemophilia in Sweden. *Haemophilia* 2004; **10**: 527–41.

7 Steen Carlsson K, Höjgård S, Lindgren A, *et al*. Costs of on-demand and prophylactic treatment for severe haemophilia in Norway and Sweden. *Haemophilia* 2004; **10**: 515–26.

8 Joshi AV, Stephens JM, Munro V, Mathew P, Botteman MF. Pharmacoeconomic analysis of recombinant factor VIIa versus APCC in the treatment of minor-to-moderate bleeds in hemophilia patients with inhibitors. *Curr Med Res Opin* 2006; **22**: 23–31.

9 Odeyemi IA, Guest JF. Modelling the economic impact of recombinant activated Factor VII compared to activated prothrombin-complex concentrate in the home treatment of a mild to moderate bleed in adults with inhibitors to clotting Factors VIII and IX in the UK. *J Med Econ* 2002; **5**: 119–33.

10 Putnam KG, Bohn RL, Ewenstein BM, Winkelmayer WC, Avorn J. A cost minimization model for the treatment of minor bleeding episodes in patients with haemophilia A and high-titre inhibitors. *Haemophilia* 2005; **11**: 261–9.

11 Astermark J, Donfield SM, DiMichele DM, *et al*. A randomized comparison of bypassing agents in hemophilia complicated by an inhibitor: the FEIBA NovoSeven Comparative (FENOC) Study. *Blood* 2007; **109**: 546–51.

12 Steen Carlsson K, Astermark J, Donfield S, Berntorp E. Cost and outcome: comparisons of two alternative bypassing agents for persons with haemophilia A complicated by an inhibitor. *Thromb Haemost* 2008; **99**: 1060–7.

13 You CW, Lee SY, Park SK. Cost and effectiveness of treatments for mild-to-moderate bleeding episodes in haemophilia patients with inhibitors in Korea. *Haemophilia* 2009; **15**: 217–26C.

60 National hemophilia databases

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Introduction

National disease registries or databases collect observational data on the prevalence, treatment, and outcome of a wide variety of diseases. Hemophilia databases have been established in many countries including the UK, Italy, Germany, France, Spain, Australia, Canada, and the USA [1–5]. Most of these databases are currently in a process of development and expansion as advances in information technology open up new possibilities [6]. There are many challenges to this development, however, including the difficulty of obtaining long-term funding and the limitations imposed by the need to comply with data protection legislation.

The UK Database is probably the original National Haemophilia Database (NHD) and was originally established in Oxford in 1968 by the UK Haemophilia Centre Doctors' Organisation (UKHCDO). This started as a disease register, registering all new diagnoses of patients with bleeding disorder and subsequently collected data on mortality, treatments used and treatment complications, such as factor VIII/IX inhibitors and human immunodeficiency virus (HIV), hepatitis C virus (HCV), and variant Creutzfeldt–Jakob disease (vCJD).

The functions of a national bleeding disorder database

Bleeding disorder databases support three basic functions: healthcare planning, epidemiologic research, and pharmacovigilance. The database collects patient demographics and observational data relevant to healthcare concerns which are specific to bleeding disorders such as the prevalence of inhibitors, viral infections, treatment intensity, and treatment outcomes. Improvement in hemophilia treatment and the impact of new medical interventions, such as prophylaxis or the use of antiviral therapy, may thus be assessed over time by clinicians and healthcare providers.

The extent to which databases can serve these functions will depend upon the completeness and accuracy of the data held, the extent of the dataset collected and the timeliness of the data collection and reporting. Where clinical service provision for patients with bleeding disorders is poorly developed, even basic disease prevalence data may be useful for planning purposes. Where hemophilia services are very well developed, however, healthcare planning requires timely, complete, and relatively comprehensive data, since the focus in such a healthcare system is usually on optimizing the quality and distribution of care and cost control and planning. Healthcare providers are very focused on hemophilia care because of the extremely high cost of the treatment and the inexorable 7–10% annual increase in treatment cost, which they wish to understand better. This focus will only increase as all healthcare systems come under increasing financial pressure. Hemophilia databases potentially have a very important role in analyzing the causes of this growth in treatment cost, in helping to optimize treatment, and in justifying current treatment practices. For this reason alone, hemophilia databases need to collect ever more clinical outcome data.

Market research and pharmacovigilance also require real-time data collection and rapid reporting. Regulators are increasingly concerned with safety issues, such as the development of factor VIII inhibitors, for which traditional safety and efficacy licensing studies are statistically underpowered. This is a generic problem for all products used for rare diseases, because large-scale trials are impractical in these conditions for reasons of cost and lack of study subjects. For that reason, new factor VIII and IX concentrates will necessarily be licensed on the condition that extended postmarketing surveillance is conducted. National databases already collect reports of adverse events in real time, and thus may have an important part to play in conducting future postmarketing surveillance studies and in pharmacovigilance in general.

Research is an important function of any disease database but, increasingly, carefully validated and comprehensive datasets, collected by protocol, are required for useful research to be conducted within a reasonable time frame. Publications from the UK National Haemophilia Database (NHD) are listed on www.ukhcdo.org. The dataset also requires regular review, altering as required to address new questions. Collaboration with other databases may be required to achieve adequate statistical power for some studies. With this objective in mind, a high degree of harmonization of factor VIII inhibitor development has been agreed between the UK,

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German, Italian, US, and Canadian databases following discussions conducted under the auspices of the ISTH Factor VIII and IX Scientific Standardisation Sub-Committee. This may permit analysis of anonymized aggregate data from several of these databases in the future. Some databases attempt a complete data collection from all patients in their country (UK, Canada, Australia, Germany, and Italy) whereas others collect only from a selection of participating centers (US database, EUHASS: European Haemophilia Adverse Event System). Reports of data collected only from interested centers may be prone to selection bias. The dataset collected by the UK NHD may be viewed on www.ukhcdo.org.

The collection of treatment data may enable the efficacy of different treatment regimens to be evaluated, but only if adequate outcome data are also collected. The collection of mortality data makes it possible to investigate the natural history of the disease and to assess the effect of improvements in care on mortality from bleeding and to evaluate the effect of treatment complications, such as inhibitors, HIV, and HCV on life expectancy. These data have also been used by patient groups campaigning for compensation.

The problem of funding

Most bleeding disorder databases were started by groups of enthusiastic clinicians with little or no funding or a short-term grant from a funding body or from one of the manufacturers. Many continued for many years with little funding, including the UK NHD. Eventually a point is reached with all such databases when the data collected by such a small operation are no longer adequate to address either healthcare planning or current research needs. At this point in its evolution, the database must either develop and expand considerably or close. This requires a lot of money and commitment. Making this developmental leap is a challenge and many databases have turned to industry for the short-term development money needed to address this challenge. Public finance may be available from government or healthcare providers. It may be argued with healthcare providers that the cost of setting up and running such a database is a very, very small proportion of the high cost of the treatment being monitored, and may help them to plan. Public funding is associated with a high level of bureaucracy and accountability, however, and this may alter the direction that the database may take. Ironically, unrestricted grants from industry usually turn out to be genuinely unrestricted and leave the database policy-makers with a free hand to take the database in the direction that they want. There is no single model for funding such a database and most continue to struggle to fulfill long-term aims with short-term funding. A recent survey conducted by the author showed that although the Italian database was funded by industry, EUHASS, the UK NHD and the US, Canadian, Australian, and German databases were all supported by public money.

Governance issues

Even though databases collect observational data only and do not conduct interventional clinical trials, they nevertheless exist in an increasingly tightly controlled regulatory environment. Ethical approval for databases is not mandatory in many jurisdictions but is recommended. Databases also have a duty to put in place appropriate mechanisms to ensure strict data security and to comply with the data protection laws. Finally, all databases require an oversight committee as a governing body to review the running of the database, consider requests for data, direct database policy, and to produce research from the database. These are discussed in greater detail below.

Data security

Modern databases store all their data electronically and all their data collection is also electronic. Data security has to be designed into such systems at a high level. Access to the database should be password protected, with timed lockouts closing down access to end-users who leave their computers unattended. Registered users with a password and username may log on to the database to interrogate it or enter data into a holding database, but may view named data on patients from their own center only and aggregate anonymous data from the rest of the country, for benchmarking comparison. Electronic communications with the database should be encrypted using a 126-bit encryption key. The UK database is also protected from the web by the firewalls of the NHS net. Named patient data are communicated only within this safe envelope and only using an encrypted link. All reports are patient anonymized.

No copies of the database or part of the database are permitted outside the database building. Daily back-ups are stored in a fireproof safe. The data are held on a file server housed in a secure, temperature-controlled server hotel. The database area is always locked when unattended. The office is largely "paperless."

Annual inspections by the Caldecott Guardian are requested by the database to document secure data handling and compliance with the Data Protection Act 1998 and the Caldecott Guardian's report is published on the UKHCDO's website (www.ukhcdo.org) to demonstrate its compliance to the outside world.

Data Protection Act

Data protection legislation must be considered and complied with when setting up a disease register. The Data Protection Act (DPA) (1998) of the UK was introduced to tighten up data protection and to harmonize with existing European Union legislation (http://www.opsi.gov.uk/Acts/Acts1998/ukpga_19980029_en_1). The Act concerns all "named" data, which is defined as data linked by *any identifier* linking the

data back to the patient's name or record. Patients have to give consent for their data to be included in a database of named data. At the present time, this does not have to be written consent and an "opt out" approach is widely used. Accordingly, in the UK, the patients are informed of the database at regular intervals using a leaflet which is posted to them and also available on our website (www.ukhcdo.org) and informed of their rights under the DPA 1998 so that they may have their data deleted or not included if they wish. Very few patients have opted out, perhaps because the database is well established and has been shown to be of benefit to patients, even in their campaigns for recompense following HCV and HIV. We have 23 000 patient records in the UK NHD, increasing at the rate of about 1000–2000 patients annually, and these patients are circulated every 5 years.

Data protection legislation has been harmonized to a high-degree in the European Union (EU), and very similar legislation exists in North America and elsewhere. Nevertheless, local variations in interpretation of the law have caused considerable problems and delays in setting up databases in some countries. Some countries have been unable to establish a national hemophilia database because local concerns about data security and data protection could not be satisfied. Even for existing databases the cost of addressing data protection legislation and the risk of patient nonparticipation was widely perceived to be a threat to future activities of such databases [6–8]. Explicit written consent may become the norm in the future, but the danger to databases of such an approach derives not just the enormous effort required from medical and nursing staff to obtain such consent but also because the commonest reason for failure to obtain consent is passive failure to fill out and return the form rather than active refusal to participate. Participation in the Canadian Stroke Registry, for example, was reduced to 39% following the need for written consent [3].

Anonymous or named database?

The UK NHD has been a named database since its inception and consequently had to fully address the provisions of successive Data Protection Acts as they came into law. The possibility of anonymizing the database was carefully considered by UKHCDO when the latest Data Protection Act came into force in 2000, but it was felt that the advantages of maintaining a named database far outweighed the effort required to obtain the patient's implied consent. Having patient identifiers serves an important function in avoiding double counting. In a densely populated country such as the UK, patients may legitimately attend two hemophilia centers working together in a hub and spoke collaboration and may have attended up to seven different centers at one time or another. Furthermore, it would have been impossible for us to conduct some of our healthcare planning functions for the Department of Health for England without recording multiple patient identifiers. The distribution of finance for the rollout of recombinant factor VIII and IX and the modeling of the National Procurement for clotting factor concentrates required the NHD to have data including the patient's name, NHS number, GP practice code, annual factor usage, brand, and unit cost.

Some databases, including the Canadian CHARMS database and the Italian database, have attempted to anonymize their data by using a patient code or number [4]. This code may qualify as a patient identifier under the definition used by the DPA, however, since the code has to be traceable back to the hemophilia center and the patient to avoid duplicate entries for patients attending more than one center. Since there is a high degree of harmonization of data protection legislation, it is possible that this approach to anonymization could be challenged under local data protection legislation. Since the use of such a patient code may not avoid the need for full DPA compliance and full patient consent, the value of this approach is, therefore, questionable.

Database oversight committee

Many, but not all, hemophilia databases are run under the umbrella of their National Haemophilia Treaters Organisation. All should be directly supervised by an oversight committee meeting regularly and reporting back to their parent organization and to other stakeholders. This oversight committee will determine database policy and direction, regularly review the dataset, oversee the organization and running of the database and produce research and reports from the data. All the stakeholder groups should be represented on this committee. The oversight committee for the UK National Haemophilia Database, for example, includes the Vice Chair and Chair of UKHCDO, the Chairs of all the UKHCDO working parties, a software engineer, a representative of the UKHCDO secretariat and representatives from the Haemophilia Nurses Association, the Data Managers Forum, and the Haemophilia Society, a patient, and two healthcare commissioners. There is also input from the Department of Health.

Technical specification, design, and staffing

The philosophy behind the organization of the database is to maximize data collection whilst minimizing duplication of effort from the hemophilia centers. Hemophilia centers already collect a great deal of data, partly for clinical management and partly for financial administration. These may be held on computer systems not compatible with the national database and, indeed, neither the US nor the Canadian databases are networked. We have developed a software system for hemophilia center administration, the Haemophilia Centre Information System (HCIS), which collects the clinical information required for patient management, the financial information required for billing, and the data required by the national database, and which interfaces both with the local hospital patient administration system (PAS) and with the national haemophilia database. Data, therefore, need to be

keyed in only once and may be exported to the national haemophilia database semiautomatically once a quarter. About 75% of UK patients with bleeding disorders have their data on an HCIS system at present.

The flow of data through the HCIS system is illustrated diagrammatically in Figure 60.1. New diagnoses and adverse events such as new inhibitors, death, or viral infection are reported as they arise through the secure network. All UK hemophilia centers are networked to the national hemophilia database, even those lacking an HCIS system. Data are imported into a holding database from the HCIS quarterly. After verification and resolution of supplementary enquiries, the data are imported from the holding to the main database and incorporated. The data are then analyzed and reports issued to stakeholders. The organization of the different elements of the system and flow of data are illustrated in Figure 60.2.

Patient home treatment data, including reason for treatment, bleed details including position and outcome, batch number, and dose, may be imported either manually or electronically using the Haemtrak system. This is an electronic patient information system sponsored by Baxter to replace the Advoy system, which was recently withdrawn from the UK. Similar systems have been developed in other countries.

The database is written in a robust program which will accommodate simultaneous multiuser access such as SQ-server or Oracle. Most national databases use such software, a small minority using Microsoft Access™, which is much less robust. The data are stored on large file servers with automatic duplication such as RAID (random array of inexpensive drives), ensuring that hard-drive failure does not lead to loss of data. There is a secure power supply ensuring that power cuts do not lead to loss of data. Tape back-ups are held in a fireproof safe.

A recent survey conducted by the author showed that database staffing varied from one (Canada) to nine members of staff for the US registry. The UK NHD has seven members of staff, including software support staff, a full-time statistician, an administrator, and three data chasers. The constant task of chasing data and verifying it is very labor intensive despite the support of an impressive hardware and software infrastructure. The staff also provide logistic support for the specific research projects of individual UKHCDO working parties.

The future

The HCIS will be networked so that all participating hemophilia centers in the UK may administer their centers and

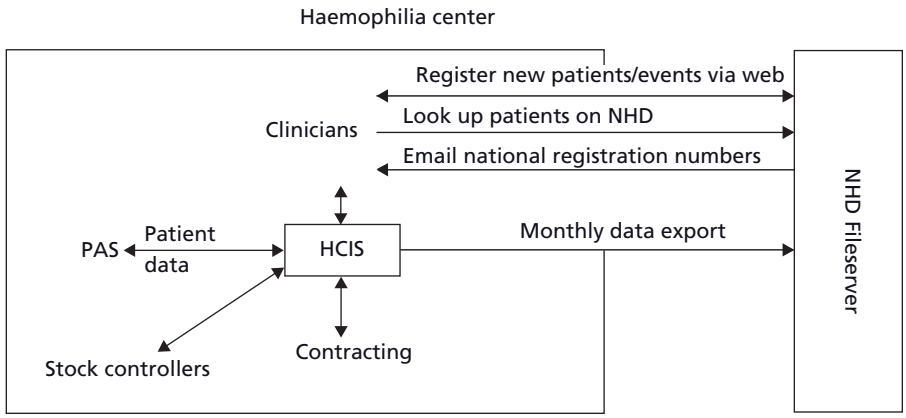

Figure 60.1 The Hemophilia Center National Haemophilia Database (NHD) net. All data sent between the center and the NHD is encrypted with 128-bit SSL security.

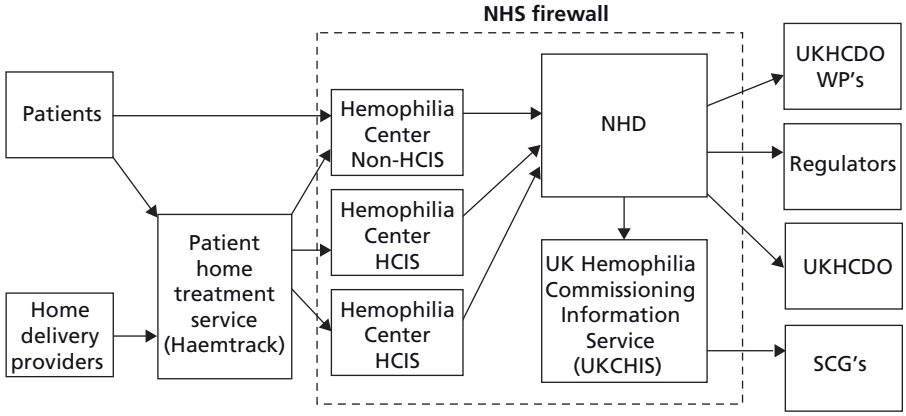

Figure 60.2 National Haemophilia Database information technology structure.

collect their data directly using two centralized file servers working in tandem rather than a server in their own hospital. Improvements in band width and speed within the health service ("Connecting for Health") have made this advance possible. This will reduce the cost of running the system, improve reliability, and make it easier to get smaller centers using the HCIS system. It will also give the national database access to far more data.

The future is also likely to see hemophilia databases maturing and developing stronger relationships with healthcare commissioners, industry, and regulators since these databases will become the natural source of data for healthcare planning, outcome evaluation, and pharmacovigilance. Databases will also become a powerful tool for large-scale epidemiologic research and the evaluation of treatment outcomes. This requires long-term planning and commitment.

References

1 Evatt BL. World Federation of Hemophilia guide to developing a national patient registry. World Federation of Haemophilia, 2005. Available at: http://www.wfh.org/2/docs/Publications/Diagnosis_and_Treatmen/Registry_Guide_2005.pdf (Accessed on 3 May 2009).

2 Hay CRM. The UK Haemophilia Database: a tool for research, audit and healthcare planning. *Haemophilia* 2004; **10**(Suppl. 3): 21.

3 Walker I. The Canadian Hemophilia Registry. *Haemophilia* 2004; **10**: 21–2.

4 Walker I, Pai M, Akabutu J, *et al.* The Canadian Hemophilia Registry as the basis for a national system for the monitoring of the use of factor concentrates. *Transfusion* 1995; **35**: 548–51.

5 Iorio A, Oliovechio E, Morfini M, Mannucci PM, on behalf of the Association of Italian Hemophilia Centre Directors. Italian Registry of Haemophilia and Alied Disorders. Objectives, methodology and data analysis. *Haemophilia* 2008; **14**: 444–53.

6 Baker RI, Laurenson L, Winter M, Pritchard AM. The impact of information technology on haemophilia care. *Haemophilia* 2004; **10**(Suppl. 4): 41–6.

61 Comprehensive care and delivery of care: the developed world

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Introduction

Historically, developments in hemophilia care have been entirely dependent on close collaboration between those who care directly for patients and their families and those from a wide range of professions and organizations supporting provision of services. Effective hemophilia therapy, until recently, was almost totally dependent on plasma-derived clotting factor concentrates, and in many countries this required detailed arrangements with national blood transfusion services and/or the purchase of concentrates from international plasma fractionators. Global issues have traumatized hemophilia like no other medical condition; the transmission of viral infections or sudden unpredictable shortages of therapeutic concentrates are but two examples. Those with responsibility for providing services, therefore, have to keep a very broad national and international perspective of factors that might influence the availability and safety of therapy.

To collaborate with other organizations, e.g., blood transfusion services, hemophilia physicians have had to develop national organizations to negotiate provision for patients. In the UK the Haemophilia Doctors' Organisation (UKHCDO) is an example of an organization that has grown from a small beginning to a larger and effective collaboration of doctors who have been able to lead effectively the developments in care and set standards to improve the lives of those with hemophilia. To help recruitment and training of physicians in the specialty, a European curriculum for thrombosis and hemostasis has been developed [1]. Similarly, patients have organized themselves into national societies, for example the UK Haemophilia Society (www.haemophilia.org.uk), and these have been very effective in patient advocacy. Many other countries have similar organizations, e.g., the National Haemophilia Foundation in the USA (www.hemophilia.org). These national societies have been drawn together into the World Federation of Hemophilia, which has promoted hemophilia care particularly effectively in developing nations (www.wfh.org). It has encouraged international collaboration between designated specialist centers of expertise (WFH International Training Centres) and countries with developing services.

The first part of the chapter outlines the overall framework within which national hemophilia services should be provided as has recently been set out by the European Association for Haemophilia and Allied Disorders (EAHAD; www.eahad.org) in the document *European Principles of Haemophilia Care* [2]. To illustrate how these principles have developed in detail to provide services, the arrangements in the UK are described in some detail as they offer one national model which has worked effectively over the past 40 years [3].

European Principles of Hemophilia Care

Guidance on how hemophilia services should be developed in Europe has been offered by the Interdisciplinary Working Group (IDWG), which comprised 45 established hemophilia physicians from 19 European countries [4]. This group compiled the *Principles of Haemophilia Care* [2] (which was subsequently reviewed, endorsed, and adopted by EAHAD). The document describes the components of a national comprehensive care hemophilia service under 10 headings (Table 61.1). These emphasize the necessity for coordination of the services, and the first recommendation states that this can best be accomplished by having a national committee to include all stakeholders to oversee and plan services. The second, and almost as important, recommendation is that there should be a confidential national registry of all patients. By knowing the number and severity of patients with congenital bleeding disorders it becomes possible to start to plan the service and especially ensure the provision of therapeutic materials. The third recommendation relates to ensuring that comprehensive care hemophilia centers and hemophilia centers are established within an appropriate network; the exact arrangements

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Table 61.1 European Principles of Hemophilia Care.

National hemophilia coordinating organizations with supporting local organizations
National haemophilia patient registry
Provision and maintenance of comprehensive care centers (CCCs) and hemophilia treatment centers (HTCs)
Partnership in the delivery of hemophilia care
Access to safe and effective concentrates at optimum treatment levels
Access to home treatment and delivery
Access to prophylactic therapy
Access to specialist services and emergency care
Management of inhibitors
Education and research

Adapted from [2] with permission.

will depend upon the distribution of patients and geographic factors. The other recommendations relate to details of service provision at the centers, e.g., monitoring of adverse therapeutic events, home therapy, and arrangements for prophylaxis. The *European Principles of Haemophilia Care* is now being used in Europe as a framework for service development throughout the continent especially in countries with less well developed nationally coordinated arrangements. It has proved useful to advocates to take to national government health departments when campaigning for improvements in services. To promote acceptance of the 10 principles of care within the European community, it has been presented to, and well received by, members of the European Parliament.

It also became clear during the discussions to develop the European Principles of Hemophilia Care that there was a need to have secure arrangements for monitoring the occurrence of adverse events, particularly in relation to therapy, in large numbers of patients to increase the likelihood of detecting, particularly infective events, early. This led to the establishment of the European Haemophilia Safety Surveillance Scheme (EUHASS) (www.euhass.org), which will record adverse events in over 15 000 patients with hemophilia. This enterprise demonstrates the very substantial benefits of international collaboration in improving safety for patients.

Arrangements for hemophilia care in the UK

Over the past 60 years the UK's hemophilia service has evolved in response to advances in medical knowledge and provision of therapeutic materials and is now one of the most carefully defined and audited national services. Since 1950 there has been a national UK approach to delivery of hemophilia care and this was initially directed by the Medical Research Council (MRC), which recognized 19 centers where special facilities were available for diagnosis and treatment of hemophilia [4,5]. These centers issued hemophilia identity cards and kept a local list of patients as well as contributing names to a central register kept by the MRC. In the decade that followed, the early freeze-dried concentrates were developed from animal and human plasma, but the major breakthrough was treatment with cryoprecipitate in 1965. In 1964, the responsibility for oversight of hemophilia passed to the Ministry of Health, which in 1968 issued a health memorandum identifying 36 centers that would diagnose and treat patients. Three of these were designated "special treatment centers" which would undertake surgery. In October of that year the directors of these hemophilia centers were invited to the opening of a new building at the Oxford Haemophilia Centre. At a party organized by Dr. Rosemary Biggs for the event it was agreed to have regular national meetings of directors and that annual treatment data would be sent to Oxford for collation. This was the beginning of what later formally became the UKHCDO. With further developments in the provision of hemophilia care, the Department of Health issued a further document in 1976 setting out revised criteria for services which should be provided at the 52 hemophilia centers. Of these, seven were designated regional hemophilia centers and the others hemophilia centers and associate centers, although the facilities which should be provided at each of these three types of centers were not specified. At this time, the collection of national data was extended to include all patients known to each center with hemophilia, and in addition treated carriers and von Willebrand disease patients. Subsequently, it extended to include all patients with von Willebrand disease. Thus, over the past 50 years there has been national coordination of hemophilia care with the arrangements having to change in response to advances in therapy as well as the way the government has overall managed the state national health service.

Current UKHCDO activities

UKHCDO's structure had to change again in 1993 when the government abolished health regions and established more "independent" trust hospitals. This led to a further development in hemophilia care arrangements with the recognition of Comprehensive Care Haemophilia Centres (CCCs). The 24 centers were so designated if they looked after at least 40 patients with severe hemophilia and offered a broad range of services as specified in a further government health circular [HSG (93) 30] [6]. In addition, there were 79 Hemophilia Centres with a responsibility to provide a more limited range of services. At this time, the constitution of the organization was revised to extend the membership from directors of centers to all doctors working within the hemophilia environment, and this was recognized by the 'D' in UKHCDO changing from "Director" to "Doctor," and thus allowing the abbreviation for the organization to remain unchanged! It is a three-tiered organization with an executive, advisory group

(of representatives of CCCs) and doctors working in hemophilia centers. The executive, or office bearers, is elected by the entire membership thus allowing more of those providing the service to be enfranchised. There is an annual meeting of all members, which often includes an educational symposium, and the advisory group meets about four times annually to direct the national hemophilia agenda.

During the past 40 years in the UK, therefore, the benefits of advances in hemophilia care have extended from the small number of the original Special Treatment Centres and many patients being treated in local district hospitals without ready access to comprehensive care, to a service with more large CCCs and Hemophilia Centres, which are able to offer a higher quality of service. The loss of Associate Centres and smaller Hemophilia Centres over the past 10 years has led to a gradual centralization of patient care in the UK. This has come with the loss of an association of an individual patient with a local hematologist and hospital, a change that was possible with the development of freeze-dried concentrates and consequently home therapy. The centers at present vary greatly in the number of patients each treats annually; more than 30 treat fewer than 10, whilst a small number serving large metropolitan populations treat over 200 (Figure 61.1).

Over the past 20 years, Scotland, Wales, and Northern Ireland have developed a degree of devolution from the UK government in London. In Scotland the healthcare provision is now a different service from England with its own funding and priorities and it is led by the Scottish Government and the Parliament in Edinburgh. To address this devolution, hemophilia directors in Scotland (along with Northern Ireland, for other historical reasons) meet regularly to promote and coordinate arrangements. This ensures that there is effective collaboration and participation by all hemophilia physicians and allows a coordinated approach to commissioners and particularly for concentrate procurement. These arrangements dovetail well with national UKHCDO, and in the future it is likely that English regions (which each have a population similar to that of Scotland) will have similar local networks.

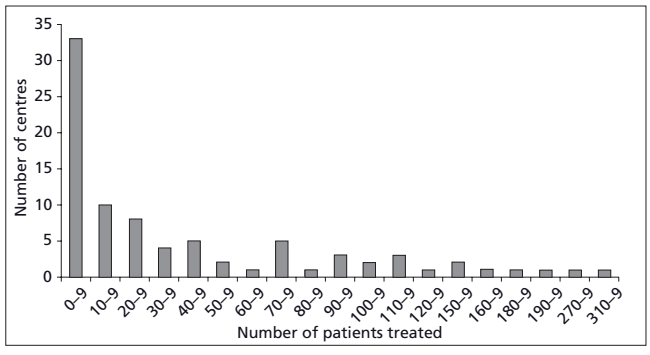

Figure 61.1 Number of patients treated at each UK Comprehensive Care Centre and Hemophilia Centre (with kind permission of UKHCDO).

Responsibilities of UKHCDO

In Britain, UKHCDO is formally defined as a charity and is subject in England to charity law and the charity commissioners. This offers it some protection from taxation of its income but restrains it from "political campaigning." This does not prevent it, however, from offering firm guidance (wherever possible, evidence-based) on matters related to provision of care for those with hemophilia particularly in relation to therapy and blood product safety. The aims of the organization are formally set out in constitution and are to promote care of those with hemophilia and related bleeding disorders, promote research, and advance education of medical and nursing staff and the general public.

The Advisory Group is the main forum for directing UKHCDO's professional responsibilities to oversee the quality of hemophilia medical care throughout the UK. This includes all the responsibilities of CCCs directly (as set out below) as well a general oversight of the large number of hemophilia centers and their activities. Although it is not managerially responsible directly for the service, this being the responsibility of the individual hospitals, it has a major commitment to inform those funding services and to work with the departments of health of the four countries in the UK. Over the years this has predominantly been with issues related to the quality of hemophilia care, the availability of therapeutic concentrates and blood safety. This has required a UK national approach partly because the issues are often technically complex, e.g., blood safety, costly and required close collaboration with the blood transfusion services in each UK country.

UKHCDO National Register of Patients

Following on from the register of patients held by the MRC, since 1969 UKHCDO has maintained a confidential national register of patients with heritable bleeding disorders (as well as acquired hemophilia and von Willebrand disease). Although initiated as a card index system, it has been computerized and is now held on a secure server that allows individual hemophilia centers to report new patients and review details of patients registered at their center. Oversight of the National Haemophilia Database (NHD) (www.ukhcdo.org) is the responsibility of the UKHCDO Data Management Group, which is convened by the organization's vice-chairman and comprises chairmen of the individual working parties, a Haemophilia Nurses Association (HNA) representative, and two patient representatives. Under the Data Protection Act 1998, patients should give informed consent to being on the register and to help the process of informing patients about the database through an information leaflet (available at www.ukhcdo.org) that has been produced and widely distributed. Patients can request a copy of their own data in the register. Each year hemophilia centers inform the database which patients have been treated and the amount of and type of products received. It also records whether the patient has

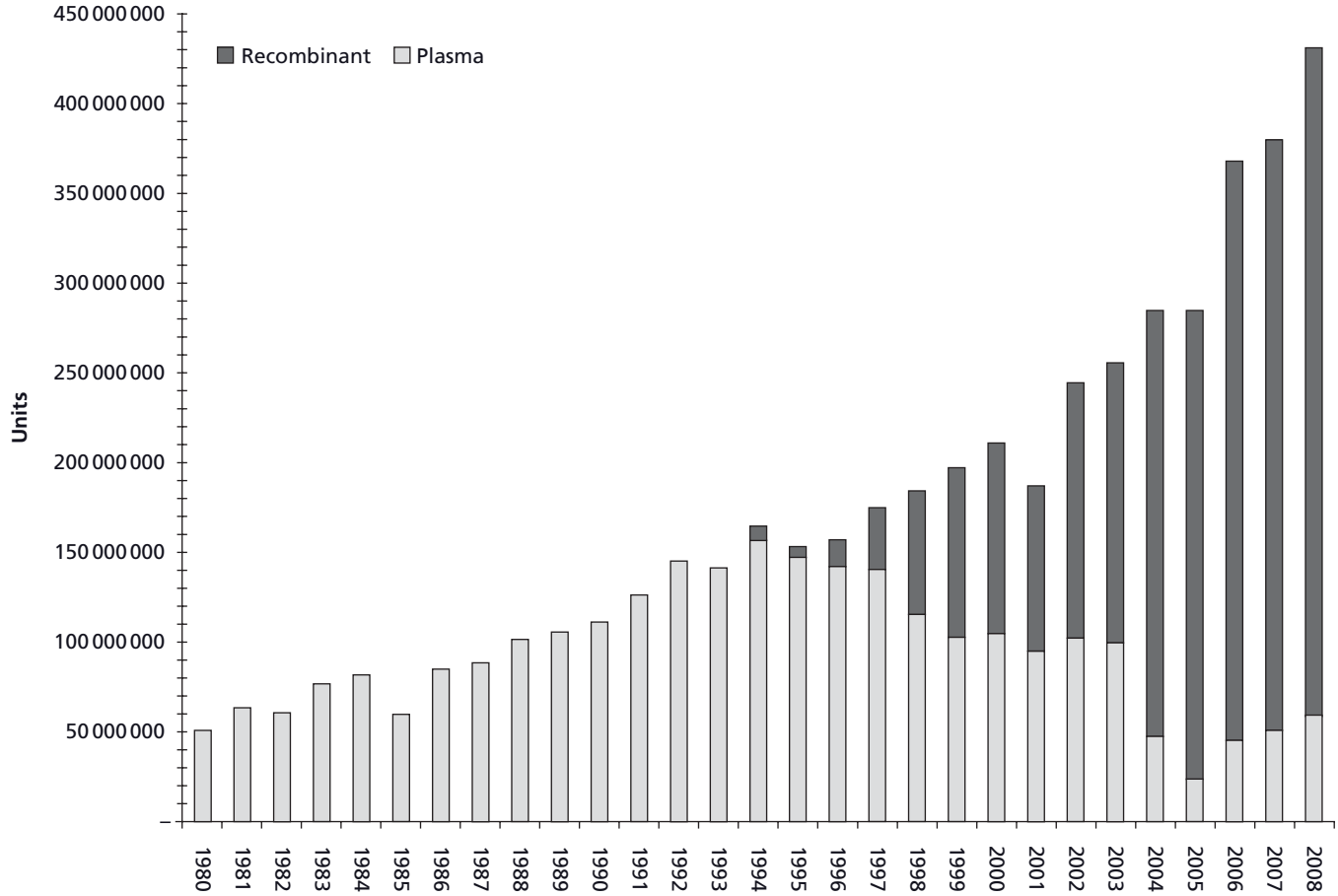

Figure 61.2 Total annual use of products used in the UK to treat hemophilia A, 1980–2008.

an inhibitor, the HIV status, and whether the genetic mutation is known (but not details—this being held at the hemophilia center which requested the investigation). This allows very accurate assessment of the national use of blood products [7], and Figure 61.2 illustrates the amount and changes in hemophilia treatment in the period 1969–2006.

The UKHCDO database has been an essential resource for planning the healthcare needs, particularly in relation to knowing the total number patients of each severity of hemophilia and the amount of concentrate used. Over the past 20 years the increase in concentrate use has been recorded at 5–10% per annum and attempts have been made in some centers and in Scotland to assess the reasons for this [8,9].

UKHCDO working parties develop guidelines

One of the other important responsibilities of the advisory group is to establish short-term working parties, which develop guidelines to inform practice and set standards (against which the quality of the service can be audited). Copies of the guidelines are available at www.ukhcdo.org. The trigger for establishment of a working party is either when new technology leads to a change in emphasis in clinical service, e.g. advances in ability to identify hemophilic mutations led to the establishment of a Genetics Working Party UKHCDO (2004), or when existing guidelines require updating, e.g., *Guidelines on Therapeutic Products* [10]. Whilst the membership of these working parties is predominantly those working directly in hemophilia care, they also offer an opportunity to include specialists in other fields, e.g., orthopedic surgery or hepatology, who offer stimulating contributions and different perspectives. As can be seen from the titles of the current working parties, listed in Table 61.2, they cover a broad range of topics which are all central to hemophilia.

Although UKHCDO's mandate is to promote hemophilia care in the UK, it does this also by active collaboration with other professional medical organizations, particularly the Royal Colleges of Physicians, Pathologists, and Paediatrics and Child Health, as well as the British Society of Haematology and the British Society for Haemostasis and Thrombosis. This is important to ensure appropriate dovetailing of hemophilia services with general healthcare arrangements and to enable the wider aspects of professional training and developments to take place within the UK medical environment.

Table 61.2 UKHCDO current working parties.

Audit
Data management group
Inhibitor
Pediatrics
Transfusion transmitted infections
von Willebrand disease
Genetics
Rare hemostatic disorders

Table 61.3 Professional organizations contributing to the Haemophilia Alliance.

UK Haemophilia Centre Doctors' Organisation
UK Haemophilia Society
UK RCN Haemophilia Nurses Association
Haemophilia Chartered Physiotherapists Association
Clinical Scientists Group
Institute of Biomedical Science
Haemophilia Social Workers Group

Haemophilia Society

The UK Haemophilia Society is an organization of patients and their families which provides support for those with heritable bleeding disorders (www.haemophilia.org.uk). It is a well-organized and effective pressure group which encourages improvements in service provision. It has a major educational responsibility and publishes highly regarded information booklets. It is an active member of the World Federation of Hemophilia (www.wfh.org).

Haemophilia Nurses Association

In parallel with the development of UKHCDO has been the emergence of organizations of other professions contributing to overall hemophilia care. Senior nurses now play a major role in both the organization of hemophilia activities as well as the day-to-day treatment of patients both in hospital and at home. In the UK the Haemophilia Nurses Association (HNA), led by an organizing committee, comprises about 150 members and is incorporate within the national Royal College of Nursing (www.rcn.org.uk). Nurses wishing to develop a career in hemophilia are now encouraged to take an advanced training course in hemophilia; these are run as part of a postgraduate studies program by several universities. Continuing professional development is through regular national educational meetings and the development of guidelines on various aspects of hemophilia care.

Haemophilia Chartered Physiotherapists Association

The Haemophilia Chartered Physiotherapists Association brings together those with skills in developing the musculoskeletal system, and thus helping to prevent bleeds and minimizing their detrimental effects when they do occur (www.cps.org.uk). Although a much smaller group than the nurses, they have been well organized nationally and have developed and published a very useful recent guide.

Social work support

Social work support is critical both to help a family adjust to a new member with hemophilia and to assist those who develop relationship (and financial) difficulties as a result of their hemophilia or the consequences of its treatment, e.g., HIV. In the UK, hemophilia social workers collaborated nationally to sustain and develop their expertise and services. With the move away from hospital-based social work services to the community it has been harder to maintain an active professional grouping, and the amount of support overall which social workers can provide has regrettably declined.

Laboratory scientists

Laboratory scientists provide the critical diagnostic services essential for hemophilia centers. Those with an academic scientific background as clinical scientists are employed primarily in research and development activities and more recently in ensuring that the latest advances in genetic techniques are available to benefit hemophilia families. As this has become one of their primary responsibilities, they have collaborated to establish the UK Haemophilia Genetic Laboratory Network and through this grouping have developed effective guidelines and standards for hemophilia genetic services (www.ukhcdo.org). Biomedical scientists, with a more technical focus on the laboratory service, are members of the Institute of Biomedical Scientists (IBMS) which is a large umbrella organization for scientists in all branches of pathology (www.ibms.org).

Haemophilia Alliance

One of the challenges of hemophilia care is to ensure that the multidisciplinary team works collaboratively within the hospital and community and to provide as seamlessly as possible the range of appropriate services. To continue to develop this integrated service the professional organizations outlined above, along with the patients' Haemophilia Society, have come together to form the umbrella organization The UK Haemophilia Alliance (Table 61.3). This has developed and published models for comprehensive care for hemophilia in its service specification, which has recently been updated (www.haemophiliaalliance.org.uk). This is now accepted as the standard of service, which should be funded by commissioners and provided by hospitals with CCCs and hemophilia centers.

Comprehensive hemophilia care in the UK

One of the guiding principles in designing arrangements for providing care for those with hemophilia and their families is that all individuals, wherever they live, should have access to the full range of services and specialties that make up comprehensive care for hemophilia. Whilst the majority who live near a CCC will be able to access these directly, those living more remotely who attend their local hemophilia center may need to be referred to the most convenient CCC for some of the more specialist aspects of clinical or laboratory service. For this to work well there should be agreed arrangements for appropriate referral of patients between hemophilia centers and the local CCC as part of a collaborative network with agreed protocols.

The range of services that contribute to comprehensive care have been set out in the 1993 health circular [HSG (93) 30] [6] and more recently in greater detail in the Haemophilia Alliance Service Specification [11].

Haemophilia Alliance Service Specification

The service specification of standards for hemophilia care was compiled by a multidisciplinary team representing the Alliance's constituent professions and the Haemophilia Society (www.haemophiliaalliance.org.uk). The standards were based on guidelines, which had been previously issued by the individual professional groups. It thus brought together into one document a description of the standards for a coordinated service for patients and their families, and in doing so provided an invaluable resource both for those funding services as well as those in the hospitals providing the care. The contents of the report are listed in Table 61.4.

Service standards and delivery

This section sets out in detail the provisions which need to be made to fulfill the requirements of the hemophilia health circulars issued by the Departments of Health for the constituent counties of the UK [6,12,13]. The importance of patients being able to choose at which CCC or hemophilia center they are registered is emphasized, as is their right of access to a CCC. The range of services which a CCC should provide are set out in Table 61.5. To provide these it will often be necessary to have network arrangements with specialists, e.g., orthopedic surgery. The details of these networks will very much depend on how the other specialists provide their services locally and of the arrangements between CCC and the local hemophilia centers. Furthermore, hemophilia centers vary greatly in the range of services they provide but as a minimum they must be

Table 61.4 National service specification for hemophilia.

Topics covered in the specification
Target patient group
Methodology
Service objectives
Service standards and delivery
Quality standards
Treatment recommendations
Carrier detection, genetic counseling, and antenatal diagnosis
Outcomes
Service arrangements
Purchase of coagulation factor concentrates
Record-keeping and data collection

Table 61.5 Functions that must be able to be carried out by comprehensive care centers.

Coordination of the delivery of hemophilia services—in both the hospital and the community—while liaising with affiliated hemophilia centers and appropriate community agencies
A 24-hour advisory and response service for hemophilia centers, general practitioners, dental surgeons, hospital doctors, patients, and families
Delivery of a comprehensive care program for patients with hemophilia; there must be at least 40 severely affected patients with hemophilia under the care of the center
A home-therapy program for patients with severe hemophilia, including the administration of prophylactic therapy where appropriate
Home treatment training programs, including home and school visits where appropriate
Provision of coagulation factor concentrates, for both hospital treatment and home-therapy programs
A diagnostic and reference laboratory service, performing a full range of laboratory tests for the diagnosis and monitoring of inherited and acquired disorders of hemostasis
Counseling for patients and their families
Social work support and welfare advice
Genetic counseling and diagnosis, in conjunction with specialized laboratories
Physiotherapy
Specialist operative and conservative dentistry
Specialist rheumatologic and orthopedic follow-up and intervention
Provision of obstetric and gynecologic support for the clinical management of hemophilia carriers and women with von Willebrand disease
Specialized services for patients with HIV and hepatitis, including support groups
Family support groups
Participation in clinical trials
Participation in clinical and laboratory audit, external and internal quality control, with submission of results to commissioning authorities
Participation in research and development
Educational programs for medical and nursing staff, biomedical scientists, and related paramedical personnel
Educational programs for patients and their families concerning all aspects of home therapy and community care

able to provide an emergency 24-hour treatment service, diagnose the commoner inherited bleeding disorders, provide advice, administer a home therapy program, and participate in appropriate audit and quality control. It cannot be emphasized too strongly that for these specialty networks of services to function effectively investment must be made to ensure that there are good and effective communications between the providers of the individual specialist services.

The service specification also emphasizes the importance of the establishment of a regional hemophilia network which comprises those who commission and fund the service as well as those who provide and use it. This regional network should oversee the coordinated service provided by CCCs and hemophilia centers to promote strategic planning and implementation of the service specification. These regional networks are at different stages of evolution, with some areas having well-developed arrangements whilst others are still being established.

Quality standards

The service specification sets out standards for data collection, laboratory performance, and clinical protocols (as set out by UKHCDO, HNA, and HCPA). Recommendations are set out for regular external audit of both CCC and hemophilia centers under the auspices of UKHCDO and HNA (see below).

Treatment recommendations

This section of the service specification covers in some detail the framework for treatment, prophylaxis and home therapy, arrangements for children, and clinical review, and includes the importance of patient participation in the care process and record-keeping. Patient treatment is guided by *Recommendations on Therapeutic Products* as issued periodically by UKHCDO [10]. Advice is given on management of inhibitors, immune tolerance, acquired hemophilia, von Willebrand disease, and rarer coagulation defects including inherited platelet disorders (www.ukhcdo.org).

Carrier detection, genetic counseling, and antenatal diagnosis

With the ability to relatively readily identify the individual mutation causing hemophilia in a family, this is now a recommended part of the assessment of hemophilic individuals. Knowledge of the mutation has made it much more straightforward to identify carriers directly rather than with the use of restriction fragment-length polymorphisms (RFLPs) to track hemophilic genes within a family. As a result of this technical advance, there is a need now to offer, to those who may be at risk, knowledge of their carrier status. This has led in the UK to a re-examination of the arrangements for genetic counseling and the establishment of local genetic registers and genetic laboratory facilities at some CCCs as set out in the UKHCDO guidelines [14]. The guidelines considered in some detail issues related to consent to genetic diagnosis both in children and adults. A patient information sheet and consent form were developed. Preimplantation genetic diagnosis has a place in hemophilia and it is likely that this will become more widely available, although probably only at a few specialist genetic centers.

Outcomes of hemophilia care

There is increasing interest in being able to assess the outcome of hemophilia therapy particularly as treatment is difficult, potentially hazardous, and expensive. Although operationally it seems most appropriate to offer "comprehensive care" at a hemophilia center rather than in a more general hematology clinic, there are relatively few studies which objectively demonstrate benefit. One such study from the USA demonstrated that patients attending a hemophilia center had a longer survival than those being seen in other clinical circumstances [15]. Outcome measurements are difficult to quantitate, particularly in the short term. One important aim of treatment is prevention of joint damage and its progression, but this needs to be assessed over many years. A recently completed randomized study has demonstrated the benefit of prophylaxis in children compared with intensive treatment of clinical bleeds [16]. However, such studies are very time-consuming and expensive and for this reason a number of surrogate measures of effectiveness of treatment have been suggested. One is the number of breakthrough bleeds per year in those on prophylaxis and another is days missed from school or work. This is a developing area of hemophilia care and one in which socioeconomic evaluation is being increasingly applied and for which appropriate tools are being developed [17,18].

Audit

Over the past 15 years in the UK it has become increasingly important to objectively demonstrate, by external review, the quality of medical services. Hemophilia has been one of the specialties in the vanguard of medical audit [19]. The initial pilot scheme was developed in 1990 in Scotland and Northern Ireland and 4 years later the scheme was extended to cover the whole of the UK every 3 years. There is a comprehensive audit proforma covering the topics listed in Table 61.6. Prior to the audit visit, questionnaires are sent to a random selection of 30 patients registered at the center, which are returned anonymously to the auditors. Two auditors, a hemophilia physician, and a nurse visit the center for a day and review the clinical and laboratory facilities. They also comment on whether recommendations in the previous audit have been implemented. Following their visit, a report is compiled and returned to the CCC. A copy is returned to the chairman of the UKCDO, to allow any unsafe arrangements or practices to be addressed urgently, but also to allow a national report to be compiled, summarizing the national service and to highlight weaknesses and areas for development.

Table 61.6 Reviewed items in the UK Haemophilia Centre Doctors' Organisation audit.

Recommendations of previous audit
Number of registered patients
Patient services at center
Coagulation factor stock control, storage, and issue
Treatment delivery
Availability of comprehensive care services
Patient medical records
Clinical governance, audit, lecture, teaching, continuing professional development, research
Pediatric care
Patient questionnaires/responses

Funding of hemophilia care

The funding arrangements for hemophilia have evolved with the development both of advances in treatment and in the way the overall healthcare budget is managed nationally. The arrangements for England, Scotland, Wales, and Northern Ireland differ in significant details. In the early days of UK plasma fractionation, to provide concentrate manufacture was funded directly by the government, but more recently in England and Wales the cost of concentrates have been "charged" to local commissioners or funders of care. Periodically, central government funds have been distributed to these local commissioners to pay for Department of Health-directed changes in treatment, e.g., provision of recombinant factor VIII and IX. Arrangements in Scotland and Northern Ireland have historically been more nationally coordinated and funded. In all countries of the UK the cost of concentrate provision is recognized as being substantial and requires explicit identification of funds by commissioners whereas the cost of staffing and most other activities of CCCs and hemophilia centers is provided from local healthcare budgets. The Haemophilia Alliance Service Specification has been very helpful in both defining and agreeing what should be provided and it is therefore of great value for commissioners to see how the overall service is configured.

Future developments in provision of hemophilia care

Over the past 20 years, factor VIII and other concentrate use has risen steadily at approximately 5% per annum, and there is no sign of this rate declining; it may in fact accelerate with increased use of prophylaxis. The cost is rising faster than the increase in use by factor VIII unitage with the move from plasma-derived to the more expensive recombinant concentrates. This financial pressure will make the gathering of outcome data to justify the increased use of concentrates more urgent. There will be an increasing focus on treatment and immune tolerance of those with inhibitors and the outcome of the international immune tolerance induction study is awaited with great interest (www.itistudy.com). Patients are seeking increasing information about their condition, and with developments in reliable genetic diagnostic tests to identify carriers there is a need for more input of time for counseling both affected individuals and family members. The aspiration that all patients should have access to the full range of comprehensive care services, wherever they live, will best be brought about with networking arrangements between CCCs and hemophilia centers. To be effective, these arrangements will need to be formalized. As ever, there will be a need to be mindful of the potential side-effects of therapy whether infectious pathogens in the concentrates or inhibitors arising secondary to their use. Those who provide hemophilia care have found the past challenging and in future it will be necessary to respond proactively to an unpredictably changing environment.

References

1 Astermark J, Negrier C, Hermans C, *et al*.; Interdisciplinary Working Group IDWG. European curriculum for thrombosis and haemostasis. *Haemophilia* 2009; **15**: 337–44.

2 Colvin BT, Astermark J, Fischer K, *et al*.; Inter Disciplinary Working Group. European principles of haemophilia care. *Haemophilia* 2008; **14**: 361–74.

3 Spooner RJD, Rizza CR. Development of a national database to provide information for the planning of care of patients with congenital blood coagulation defects. In: Rizza Cr, Lowe G (eds.) *Haemophilia and Other Inherited Bleeding Disorders*, London: Saunders, 1997: 433–53.

4 Ludlam CA, Mannucci PM, Powderly WG; European Interdisciplinary Working Group. Addressing current challenges in haemophilia care: consensus recommendations of a European Interdisciplinary Working Group. *Haemophilia* 2005; **11**: 433–7.

5 Tansey WM, Christie DA. *Haemophilia: Recent History of Clinical Management*, volume **4**. London: Wellcome Trust, 1999.

6 UK Department of Health. Health circular HSG (93) 30. Provision of Haemophilia Treatment and Care.

7 Rizza CR, Spooner RJ, Giangrande PL, UK Haemophilia Centre Doctors' Organisation (UKHCD). Treatment of haemophilia in the United Kingdom 1981–1996. *Haemophilia* 2001; 349–59.

8 Ludlam CA, Lee RJ, Prescott RJ, *et al*. Haemophilia care in central Scotland 1980–94. Demograph characteristics, hospital admissions and causes of death. *Haemophilia* 2000; **6**: 494–503.

9 Yee TT, Beeton K, Griffioen A, *et al*. Experience of prophylaxis treatment in children with severe haemophilia. *Haemophilia* 2002; **8**: 76–82.

10 Keeling D, Tait C, Makris M. Guideline on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders. A United Kingdom Haemophilia Center Doctors' Organisation (UKHCDO) guideline approved by the British Committee for Standards in Haematology. *Haemophilia* 2008; **14**: 671–84.

11 Haemophilia Alliance. A National Service Specification for Haemophilia and Related Conditions, 2nd edn. London: The Haemophilia Alliance, 2006.

12 UK Ministry of Health. Health memorandum. Arrangements for the Care of Persons Suffering from Haemophilia and Related Disorders, 1968.
13 UK Department of Health. Health circular HC (76) 4. Organisation of Haemophilia Centres.
14 Ludlam CA, Pasi KL, Bolton Maggs P, *et al*., on behalf of UK Haemophilia Doctors' Organisation. A framework for genetic service provision for haemophilia and other inherited bleeding disorders. *Haemophilia* 2005; **11**: 145–63.
15 Soucie JM, Nuss R, Evatt B, *et al*. Mortality among males with hemophilia: relations with source of medical care. Hemophilia Surveillance System Project Investigators. *Blood* 2000; **96**: 437–42.
16 Manco-Johnson MJ, Abshire TC, Shapiro AD, *et al*. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; **357**: 535–44.
17 Nicholson A, Berger K, Bohn R, *et al*. Recommendations for reporting economic evaluations of haemophilia prophylaxis: a nominal groups consensus statement on behalf of the Economics Expert Working Group of The International Prophylaxis Study Group. *Haemophilia* 2008; **14**: 127–32.
18 Schramm W, Royal S, Kroner B, *et al*.; for the European Haemophilia Economic Study Group. Clinical outcomes and resource utilization associated with hemophilia care in Europe. *Haemophilia* 2002; **8**: 33–43.
19 Lowe GDO. Clinical audit of haemophilia care. In: Rizza CR, Lowe G (eds.) *Haemophilia and Other Inherited Bleeding Disorders*. London: Saunders, 1997: 481–92.

62 Comprehensive care and delivery of care in hemophilia: the developing world

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Introduction

The management of patients with hemophilia, particularly those with severe disease (factor activity <1%), is more complex than replacement of factor concentrates alone. Since the clinical impact of the severely compromised hemostasis is felt from a very early age and the fact that optimal curative treatment still eludes this condition, these patients develop many complications that require involvement of a variety of healthcare personnel. These include the following:

- Early recognition of the condition and accurate diagnosis by the primary physician before any serious complication occurs as a result of hemorrhage.
- Consultation with a specialist physician/hematologist to plan the management of the individual including a plan for factor replacement therapy and other supportive measures.
- Counseling of the family on the implications of coping with this diagnosis and its socioeconomic impact. This usually involves a nurse or a social worker.
- Involvement of a physical therapist, physiatrist, and an orthopedic specialist to manage the almost inevitable musculoskeletal complications.
- A dentist for dentition-related complications, which are not uncommon.
- An appropriate molecular genetics laboratory for carrier detection and prenatal diagnosis, if needed.
- Infectious disease specialists and hepatologists to cope with the legacy of viral infections with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) from factor concentrates used in the 1980s among many adult patients.
- Inhibitors to factors VIII and IX develop in a proportion of these patients and require special management.

Such an approach to the management of persons with hemophilia (PWH) is termed "comprehensive care" [1,2]. The actual delivery of such care to a person with hemophilia and his family requires a healthcare system and other supportive mechanisms. This chapter will describe the special considerations required in providing comprehensive care to people with hemophilia in developing countries.

The developing world and its problems with hemophilia care

Of the estimated 400 000 PWH in the world, about 80% live in developing countries, reflecting the overall distribution of population in the world in 143 of the 191 member states of the World Health Organization [3]. As opposed to a per capita GDP of over $20 000 in developed countries, the per capita GDP in developing countries varies from less than $1000 for about 40% of these people (low income) to $1000–3000 for another 40% (low-middle income) and $3000–7000 for the remaining 20% (middle income) [4]. The expenditure on health in these countries is usually between 1% and 3% of GDP, most of which goes toward maintaining infrastructure. The limited healthcare budget under these circumstances is therefore often directed toward nutritional and infectious diseases (high-volume, low-cost conditions) rather than hemophilia or other hereditary bleeding disorders (low-volume, high-cost conditions). While there is no doubt that limited resources impact very significantly on the ability of countries in the developing world to spend on hemophilia care, the other most important factor in this regard is the attitude of the people and their government toward health care in general and hemophilia in particular. Within the social and economic diversities of these countries, examples abound of countries with similar per capita GDP spending very differently on health.

Comprehensive care and delivery of care

Providing comprehensive care to PWH in developing countries requires a few core components to be established [5]. These include:

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1 appropriate medical infrastructure;
2 identification and registration of people with hemophilia;
3 selection of appropriate models of care—protocols and products;
4 educating patients and families about hemophilia care;
5 improving social awareness of hemophilia and promoting advocacy;
6 developing a program for delivery of care.

Establishing appropriate medical facilities

There should be at least one center in each country that can provide comprehensive care according to international standards. Therefore, it is essential to identify and train physicians who are committed to the field of hemostasis. They can then help train others in the country. More emphasis should be placed on the management of bleeding disorders, indeed hematology in general, in the medical curriculum in developing countries. Annual workshops held in different regions, to emphasize various aspects of hemophilia care, can significantly improve the understanding and skills of the care providers. In this regard, programs of the World Federation of Hemophilia (WFH), such as the International Hemophilia Training Centers fellowships, center twinning program, and workshops, have been very useful in rapid transfer of information and expertise [6,7].

The number of care centers required in each country will depend on the geographic distribution of the patient population. The facilities at each center will vary according to the level of expertise and infrastructure available (Table 62.1). Detailed guidelines should be prepared for the management of these conditions in a way that is appropriate and practical for each country. It would be best to integrate these services with the existing healthcare system, if possible, so that the diagnostic and clinical facilities at these centers will be useful for patients with other bleeding disorders as well.

While the treatment of hemophilia is extremely expensive, prevention is not. This is an aspect of hemophilia care that needs particular emphasis in developing countries. With the knowledge of the genetics of hemophilia and utilizing current techniques of molecular biology, highly accurate carrier detection and prenatal diagnosis can be established cost-effectively in many of these countries [8].

It is also important that, together with help for establishing diagnostic and treatment facilities, concepts of quality management in all aspects of the work involved be emphasized. A system of clinical audit should also be developed. All laboratories should be encouraged to participate in at least one external quality assessment program [9].

Identification and registration of people with hemophilia

In developing countries from which data are available, the proportion of the estimated number of PWH identified varies

Table 62.1 Establishing facilities at different levels of care for people with hemophilia in developing countries.

Level of clinical care facilities available
Primary care center
Provision of basic care to patients with diagnosed bleeding disorders
Storage and administration of therapeutic products
Participation in appropriate clinical audit
Treatment center
All facilities mentioned above along with laboratory service for screening tests for the diagnosis of bleeding disorders. Facilities for assays and screening for inhibitors, if possible
Physiotherapy
Counseling and advisory services
Advice on home therapy, where appropriate
Comprehensive care center
All facilities mentioned above and:
24-hour clinical service capable of handling emergencies and advising other centres
Laboratory facilities for assays of factor levels and inhibitors
Specialist service for surgeries, infectious diseases, and social issues
Rehabilitation services
Reference center
All facilities mentioned above and:
Reference laboratory for evaluation of atypical cases and rarer bleeding disorders
Genetic evaluation, carrier detection, and antenatal diagnosis
Training of members of the comprehensive care team
Maintain national registry
Conduct data analysis and clinical audit
Formulation of national policies
Research that is appropriate for the country
Level of laboratory tests available
Coagulation laboratory
Blood film, platelet count, clot retraction, bleeding time, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), correction studies with "control" and factor VIII- and factor IX-deficient plasma from appropriately screened patients and inhibitor screening, qualitative test for factor XIII
Comprehensive coagulation laboratory
All tests mentioned above and factor assays (VIII, IX, I, II, V, VII, X, XI), inhibitor assays, platelet function tests, von Willebrand factor (VWF) activity
Reference coagulation laboratory
All tests mentioned above and VWF multimers, VWF antigen, factor IX antigen, genotypic analysis, carrier detection, prenatal diagnostic tests, and evaluation of rarer coagulation disorders
Coordinate external quality assessment program
Appropriate research

between 10% and 80%. Overall, only about 30% of PWH estimated to exist in these countries have been registered [10]. Inadequacy of healthcare facilities, lack of adequate knowledge of bleeding disorders among primary care physicians, and poorly developed hematology services, particularly with respect to diagnosis of bleeding disorders, contribute to the fact that the majority of PWH in these countries remain undetected or inadequately diagnosed [5].

The challenge of detecting affected people and making an accurate diagnosis of hemophilia in developing countries needs to be met at different levels—educating healthcare personnel, increasing awareness in society, establishing laboratories capable of performing tests of hemostasis (with appropriate quality control of these tests), and monitoring of these services for their impact on hemophilia care.

The importance of creating a national registry of PWH cannot be overemphasized. This is the only way to chart out the demography of PWH in any country, document their clinical status, and monitor their progress over a period of time to assess the efficacy of the care program.

Selecting appropriate models of care

Protocols for treatment

Factor replacement therapy in hemophilia is often based on following convention rather than evidence for optimum protocols. In situations without significant constraints on resources, the guiding principle is to use high doses that guarantee efficacy, albeit at an extremely high cost. In developing countries, this approach is impractical and requires a prudent selection of protocols that are most cost-efficient, since >90% of the cost of hemophilia care is the purchase of factor concentrates. Therefore, selecting suitable protocols for use in each country becomes critical [11].

The three main indications for factor replacement in hemophilia are:

1 prevention/treatment of hemarthroses;
2 surgery;
3 immune tolerance therapy.

Prevention/treatment of hemarthrosis

The predominant cause of morbidity in hemophilia is the damage resulting from repeated bleeding into joints. It has been the aim of therapy to establish a standard where damage to the joints, clinically and radiologically, can be completely prevented. This has been achieved by prophylactic replacement of factor concentrates 2–3 times/week at 20–40 IU/dose. The effectiveness of this approach in preserving joint integrity has been established with long-term follow-up data from Sweden [12]. Unfortunately, the annual cost of such therapy, currently at $100 000–300 000 per person, has been so high that it has been difficult even for countries with developed economies to adopt it universally [13]. With cost constraints in developing countries, the aim of replacement shifts from maintaining perfect joint integrity to maintaining reasonable joint function that will allow the person to remain functionally independent. This can usually be achieved with much smaller amounts of factor concentrates. Out of necessity and not out of choice, people with hemophilia in developing countries and their physicians have to accept this fact [14].

Prophylaxis in the classic way has generally been considered feasible after a usage of about 3 IU/capita is achieved. This model presumes an individual dose of 25–40 IU/kg every time. It is possible that lower doses may also be effective, or at least better than on-demand therapy. With many developing countries moving into the 1–3 IU/capita range of usage of clotting factor concentrates [15], other models of prophylaxis need to be considered until such a time as the optimal doses are worked out and availability improves in these countries. A hypothetical situation is therefore described below. Based on the usually quoted number of 5 PWH/100 000 population, a country of 10 million people will have about 500 PWH. If they have access to even 1 IU/capita, then they have 10 million units of concentrate available. This would amount to about 20 000 IU every year for each person with hemophilia. If the average weight of young children (<10 years) is taken to be about 25 kg, still the children can be given a prophylaxis of 250 IU (~10 IU/kg) two times a week, they would require about 25 000 IU for the year. It is very likely that this regimen will be better than on-demand therapy and should be attempted. Depending on the availability of concentrates over 1 IU/capita, a suitable regimen for prophylaxis can be worked out increasing the dose and frequency while all the time documenting bleeding and musculoskeletal outcome until what is acceptable to the community is achieved.

The actual amount of factor replacement for joint bleeding in developing countries is variable. The limited data available suggest that the total quantity of factor concentrate used varies from about 2000 to 30 000 IU/person annually [16]. Some of these centers that use factor concentrates in the intermediate range report preservation of reasonable joint function and functional independence. However, this is not backed by data on long-term orthopedic outcome. Therefore, it would be useful to longitudinally evaluate a large number of patients with emphasis not only on their clinical and radiologic scores but also on their functional status. Such data could help establish the dose at which cost–benefit ratio is the highest.

Surgery

Large quantities of factor concentrates are needed for PWH undergoing surgery. When factor concentrates are used at the usually recommended dosage for intermittent bolus infusions, most major surgical procedures require about 1000 IU/kg per procedure [17]. With continuous infusion of factor concentrates, this can be reduced to about 400–500 IU/kg per procedure [18]. In situations of extreme resource constraints, lower doses, aimed at maintaining 30–40% trough levels in the first

2–3 days, followed by 20–30% in the next 3–4 days and 10–20% during the subsequent days, can reduce factor usage to about 300 IU/kg per procedure even with intermittent infusion protocols for major surgical procedures, not including joint replacement surgery [19].

Immune tolerance therapy

Extremely large quantities of factor concentrates are required for immune tolerance therapy for PWH who develop persistent high-titer inhibitors. The optimal dose remains to be defined and varies. Usually, about 50–200 IU/dose, 2–3 times/week is administered for several months [20]. Very few data are available on such therapy from developing countries. In Turkey, four out of seven patients underwent successful immune tolerance therapy with 25 IU/kg factor VIII three times a week over 1–4 months [21,22]. These are encouraging data, but need to be attempted on larger numbers of patients. Unfortunately, most centers in developing countries are unable to offer such treatment for lack of resources.

Products for treatment

The greatest challenge for those attempting to provide care for people with hemophilia in developing countries is the provision of factor concentrates in adequate quantities for replacement therapy. Availability and cost determine the choice of products for factor replacement in developing countries, unlike developed countries where safety and purity are the predominant considerations. Different models and possibilities exist and each country needs to choose its options carefully [23].

Import of factor concentrates

Import of the required quantities of safe virus-inactivated factor concentrates of a suitable purity from the international market is an option. The advantage of this approach is that safe factor concentrates can be immediately made available to PWH. With the use of recombinant factor concentrates in developed countries, safe plasma-derived concentrates are now available at about $0.20–0.30/IU. Unfortunately, even at this price, they remain out of reach of most PWH in developing countries. The other important concern is that as more PWH become identified in these countries, there will not be enough plasma-derived concentrates with current levels of production to meet the needs of PWH around the world. There is need, therefore, for different levels of self-sufficiency in plasma and plasma products in developing countries.

Local self-sufficiency of plasma and factor concentrates

There are two separate issues here.

Self-sufficiency in plasma

This is certainly desirable, and requires improving and expanding blood transfusion services so that adequate quantities of safe plasma can be collected. Such plasma could be used as fresh-frozen plasma (FFP) or cryoprecipitate initially, with viral inactivation, if possible, until such a time when other options become available. If enough plasma can be collected, then fractionation can be considered.

Self-sufficiency in fractionation

Two options exist for fractionation of locally collected plasma—contract fractionation outside the country at a suitable facility or establishing infrastructure for fractionation locally. Though a variety of factors need to be considered, including volume of plasma available, quantity and purity of factor concentrates required, and the resources available for choosing between these options, it may be best for smaller countries to opt for contract fractionation and those with large populations to choose the latter option.

Perhaps the best option is a combination of these two approaches. Initially, a country could import modest quantities of factor concentrates that can provide the existing PWH with a safe therapeutic option while trying to establish a good transfusion service for collecting large quantities of plasma. As plasma collection increases, fractionation could be done either locally or on contract at a distant site to different levels of purity as deemed appropriate and factor replacement practices could change accordingly. Such an approach is also likely to be more acceptable for governments that may prefer to use their resources to support local industry rather than paying to import factor concentrates.

There are a few examples of plasma fractionation facilities in developing countries. Brazil, Cuba, Thailand, and South Africa have been able to produce small quantities of low/intermediate-purity virus-inactivated products in modest plasma fractionation plants in the past, which has served their needs to some extent [23]. In South Africa, the needs of about 1500 people with hemophilia using about 12 000 IU each annually has been almost entirely met from these manufacturing units. Large quantities of factor concentrates and other plasma products are fractionated at multiple facilities in China [24]. While developing countries attempt to establish facilities for fractionation, the over-riding principle should be attention to current good manufacturing practices (cGMPs) with regard to quality of plasma and viral inactivation.

Educating patients and families about hemophilia

While this is extremely important everywhere, it is even more so in situations where care is inadequate. A knowledgeable patient can actually help prevent iatrogenic complications of hemophilia that are not uncommon under these conditions. Facilities should be established for adequate counseling and

education about the disease for families with individuals diagnosed to have hemophilia. Apart from information related to the principles of managing this condition, they should also be made aware of the support systems available to cope with it socially and financially. Written information should also be provided to them. It would be very useful if, at the time of diagnosis and registration, an information booklet in the local language is given to each patient and his or her family. This would not only provide essential information immediately, but would also compensate to some extent for lack of proper counseling facilities at the center where the diagnosis was made. It could later be supplemented by discussions regarding specific problems.

Improving social awareness and providing advocacy

Increasing social awareness of hemophilia is important for two reasons. First, it helps identify more people affected by this condition as families with individuals who may have hemophilia seek medical attention. It also helps in creating social support for the cause of hemophilia, which can play a crucial role in improving care for PWH. Both the print and visual media can be used to achieve this.

Experience in the world has shown that getting support from government for hemophilia care requires strong advocacy groups. Establishing a vibrant and well-informed national organization that represents all PWH in a country and advocates their cause is extremely important for this. The WFH also has major programs to assist in this process.

Developing a program for delivery of care

Two models currently exist in many developing countries. The first involves support from the government and a program of care integrated with national healthcare facilities. The level of support from government and insurance agencies varies in different countries. The second is a situation where there is no significant support for PWH from the government and most of the care is provided by a parallel system of health care involving private hospitals and other nongovernmental organizations.

The data collected by the WFH through its global survey confirms that countries with successful programs for hemophilia care have support from their national healthcare systems. These are countries with GDP in the middle and low-middle income groups and strong patient advocacy groups. On the other hand, countries with the lowest GDP and insignificant patient advocacy have extremely inadequate or absent organized care for hemophilia [25]. Lobbying for support from the health budget of the country therefore becomes crucial for successful implementation of such programs and improving awareness of hemophilia and its management are crucial aspects of establishing care and its delivery.

Conclusion

The majority of people with hemophilia in the world continue to have inadequate care because of paucity of resources and lack of knowledge. Varying conditions that prevail in developing countries make it difficult to recommend an ideal model for the delivery of hemophilia care. It is possible, though, to define the basic requirements that are necessary to achieve this as described in this chapter. The details will depend upon local circumstances, and each country will need to choose the model best suited for its purpose. However, in the last decade, mainly owing to the efforts of the WFH and the cooperation of many governments, significant progress has taken place in improving hemophilia care in developing countries. If this momentum is sustained, indeed accelerated, then the quality of life of PWH in developing could vastly improve in the next decade.

References

1 Kasper CK, Mannucci PM, Boulyzenkov V, *et al*. Haemophilia in the 1990s: principles of treatment and improved access to care. *Semin Thromb Hemost* 1992; **18**: 1–10.
2 Berntorp E, Boulyzenkov V, Brettler D, *et al*. Modern treatment of haemophilia. *Bull WHO* 1995; **73**: 691–701.
3 Human Development Report, 2003. Geneva: United Nations Development Program.
4 World Bank. World Bank Report, 2000.
5 Srivastava A. Delivery of haemophilia care in the developing world. *Haemophilia* 1998; **4**(Suppl.2): 33–40.
6 Giangrande PL, Mariani G, Black C. The WFH Hemophilia Center Twinning program: 10 years of growth, 1993–2003. *Haemophilia* 2003; **9**: 240–4.
7 Carman CJ. Developing and maintaining of hemophilia programs in developing countries. *Southeast Asian J Trop Med Public Health* 1993; **24**(Suppl. 1): 46.
8 Jayandharan G, Shaji RV, Chandy M, Srivastava A. Identification of factor IX gene defects using a multiplex PCR and CSGE strategy——a first report. *J Thromb Haemost* 2003; **1**: 2051–4.
9 Preston FE. Laboratory diagnosis of hereditary bleeding disorders: external quality assessment. *Haemophilia* 1998; **4**(Suppl. 2): 12–18.
10 World Federation of Hemophilia. Global Survey, 2001.
11 Srivastava A. Factor replacement therapy in haemophilia—Are there models for developing countries? *Haemophilia* 2003; **9**: 391–6.
12 Nilsson IM, Berntorp E, Lofqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. *J Intern Med* 1992; **232**: 25–32.
13 Manco-Johnson MJ, Abshire TC, Shapiro AD, *et al*. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; **357**: 535–44.
14 Srivastava A. Choice of factor concentrates for haemophilia: a developing country perspective. *Haemophilia* 2001; **7**: 117–22.
15 World Federation of Hemophilia. Report on the Annual Global Survey, 2007.
16 Srivastava A, Chuansumrit A, Chandy M, Duraiswamy G, Karabus C. Management of haemophilia in the developing world. *Haemophilia* 1998; **4**: 474–80.

17 Rickard KA. Guidelines for therapy and optimal dosages of coagulation factors for treatment of bleeding and surgery in haemophilia. *Haemophilia* 1995; **1**(Suppl. 1): 8–13.
18 Martinowitz U, Schulman S, Gitel S, Horozowski H, Heim M, Varon D. Adjusted dose continuous infusion of factor VIII in patients with haemophilia A. *Br J Haematol* 1992; **82**: 729–34.
19 Srivastava A, Chandy M, Sunderaraj GD, *et al*. Low dose intermittent factor replacement for post-operative haemostasis in haemophilia. *Haemophilia* 1998; **4**: 799–801.
20 DiMichele D. Immune tolerance therapy dose as an outcome predictor. *Haemophilia* 2003; **9**: 382–6.
21 Kavakli K, Gringeri A, Bader R, *et al*. Inhibitor development and substitution therapy in a developing country: Turkey. *Haemophilia* 1998; **4**: 104–8.
22 Chuansumrit A, Pakakasama S, Kuharthong R, *et al*. Immune tolerance in a patient with haemophilia A and high titre inhibitors using locally prepared lyophilized cryoprecipitate. *Haemophilia* 2000; **6**: 523–5.
23 Bird A, Isarangkura P, Almagro D, Gonzaga A, Srivastava A. Factor concentrates for haemophilia in the developing world. *Haemophilia* 1998; **4**: 481–5.
24 The World Federation of Hemophilia's third global forum on the safety and supply of hemophilia treatment products, 22–23 September, 2003, Budapest, Hungary. *Haemophilia* 2004; **10**: 290–4.
25 Evatt BL, Robillard L. Establishing haemophilia care in developing countries: using data to overcome the barrier of pessimism. *Haemophilia* 2000; **6**: 131–4.

63 Comprehensive care and delivery of care: the global perspective

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Comprehensive care in the developed world

A discussion of global comprehensive care must include consideration of at least two different cycles of healthcare development—one for developed countries and one for countries with emerging economies. Prior to the 1960s, no comprehensive care was available and individuals with hemophilia suffered much the same fate throughout the world. Life expectancy was less than 20 years and severe disabilities because of joint disease developed by early teenage years [1]. In the developed countries, hemophilia associations were established for the purpose of recruiting the donors needed to supply blood to be used for hemophilia patients. The discovery of cryoprecipitate in 1964 and subsequent development of clotting factor concentrates dramatically increased the options of clinical management for patients [2]. Concentrates could be easily stored, administered at home, and carried with patients during travel. These qualities allowed early treatment of bleeding episodes, reducing pain and often the time to subsequent return to school or work, and home therapy quickly evolved as a management option. Its increasing popularity necessitated the training and education of patients about disease management. Soon, specialized centers began to emerge that delivered a number of services including home care and patient education [3–5]. Studies conducted by these centers demonstrated a profound effect produced by these approaches on patient care, survival, and general health. As a result, patient associations and physicians urged and received government support for national programs (predominantly in Europe, North America, Australia, New Zealand, and Japan) consisting of networks of hemophilia treatment centers (HTCs). These centers, made possible because the advanced economic condition of these countries, provided comprehensive services consisting of hemophilia care, orthopedic and dental services, and education and psychosocial support [6–8].

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Comprehensive care in hemophilia was indeed an early forerunner of chronic disease management, a model of care now well established in public health out-of-hospital programs.

Today, the comprehensive care model in developed countries continues to prove itself superior to unorganized care for both survival and effective utilization of healthcare resources [6].

Recent studies on over 3000 patients with hemophilia in the USA examined mortality and hospitalization rates of patients receiving care in comprehensive care centers or alternatively by private physicians. Both patient groups had similar access to clotting factor and healthcare resources. Although the most severely affected patients with the most complications were those cared for by the comprehensive care centers, they had lower mortality rates and lower hospitalization rates than those cared for by private physicians (Figure 63.1) [6,7]. Important elements of this effect were the availability of expertise for serious complications, home therapy, and consistent education of patients about their diseases—all standard services at the comprehensive centers.

The net effect is that the comprehensive care model has normalized life for patients with hemophilia in countries of the developed world. Life expectancy is approximately 65 years, serious blood-borne infections have not been transmitted by concentrates since 1990, and joint disease produced by bleeding episodes is virtually nonexistent in children under the age of 15, particularly when they are receiving prophylaxis [6,9–12]. Individuals with hemophilia pursue life with the vigor of any in the normal population.

As a result, the success of HTCs in improving health care has had a secondary effect of reducing the need for the services provided in a number of countries, particularly those on site at the centers. Surveys of HTCs in the USA have shown the average number of patient visits per week to HTCs has dropped to 5.2, only 69% of the staff time is spent on hemophilia care, and physicians in the clinics are spending only 20% of their time taking care of hemophilia patients [13,14]. As a result, training of young physicians to take care of patients with hemophilia has decreased and an impending

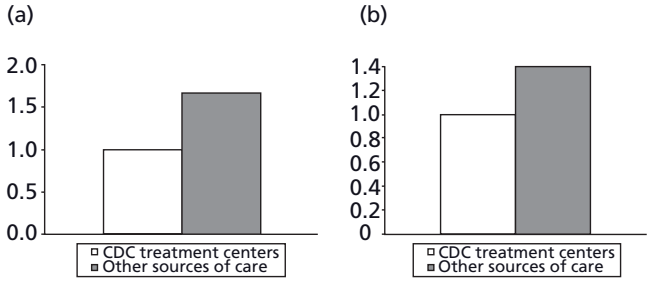

Figure 63.1 Outcomes for hemophilia treatment centers, 1993–1995. (a) Relative mortality. (b) Relative number of hospitalizations. Patients receiving hemophilia care outside the hemophilia treatment centers have 67% higher mortality rates and 40% higher hospitalizations for a bleeding complication [6–7]. CDC, Centers for Disease Control and Prevention.

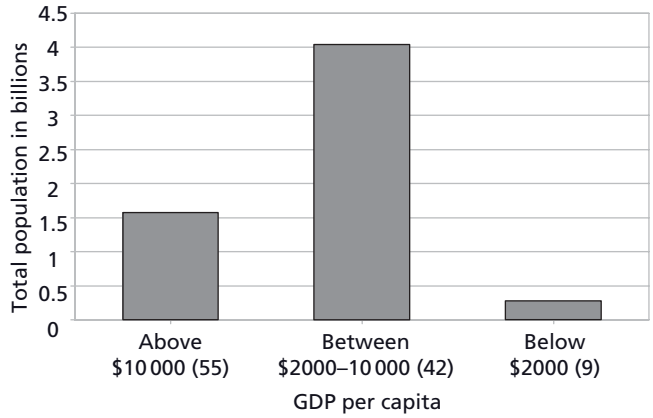

Figure 63.2 Bleeding disorders population in countries with different levels of economic development [28]. Total bleeding disorder populations by economic category (2007 data: 106 countries reported).

crisis identified as HTCs try to replace retiring physicians but trained personnel are unavailable [15]. Consequently, the work patterns and patient mix of HTCs is changing. Services are expanding to include those for women with bleeding and for people with clotting disorders, a rapidly expanding population of persons with newly diagnosed thrombophilia. In some HTCs, these patients already comprise as much as 80% or more of the clinic population, and their presentations have the result of justifying and maintaining services for hemophilia patients [Centers for Disease Control and Prevention (CDC), unpublished data].

Comprehensive care in the developing world

Current status of comprehensive care in developing countries

Establishing comprehensive care in the developing world has often remained an elusive dream.

Extensive information concerning the status of comprehensive care exists mostly for Europe, North America, Australia, and New Zealand. Excellent HTCs have been developed in a number of other countries, with the hope that longer term national networks of centers will follow. Information has been published recently on a few countries that are developing or completing successful hemophilia programs, but in most of the developing countries standards of care similar to those in developed countries are generally lacking and services vary widely depending upon availability of resources [16–20].

The World Federation of Hemophilia (WFH) maintains a database containing information on the status of care in a large number of countries throughout the world. In 1998, the WFH began collecting demographic information and health outcome data for hemophilia patients living in its member countries. These data are collected annually from most of its member organizations in 113 countries, representing 89% of the world's population. The information is used to monitor the number of people with bleeding disorders and the level of care provided to determine priorities and assist in directing the WFH's developing country programs. Termed the World Federation of Hemophilia's Global Survey, the database is one of the tools used to evaluate the level of care in various countries targeted for program development [21–28].

According to these data, the level of services available among the different countries vary widely, and are usually related to the economic capacity of the country as reflected by the per capita gross domestic product (GDP) [29]. Countries may be grouped into three categories of GDP: <$2000, $2000–10 000, and >$10 000. Developed countries generally have GDPs >$10 000 per capita per annum and almost always have fully fledged comprehensive care programs. Some of the countries in the $2000–10 000 per capita per annum group, representing the largest total population, have established or are developing comprehensive care programs. However, the third group, <$2000 per capita per annum, has the greatest economic challenges and the lowest level of services generally (Figure 63.2). The relationship of GDP to available health resources is dramatic. In the typical country with the highest GDP, adequate hemophilia care costs approximately 2–3 times the health resources available to the average inhabitant (based on the assumption that 30 000 units/year/patient is needed for adequate care). In many countries with the lowest GDP, adequate care would cost 500–700 times or more of the health resources available to the average inhabitant. Graphically, this can be expressed as the amount of a country's total health expenditures that would be needed to provide what the developed countries consider as adequate health care (Figure 63.3). There has been a slow but steady progress in product provided in the $2000–10 000 GDP economic group (Figures 63.4 and 63.5). This progress is likely a combination of improving economies combined with the development of

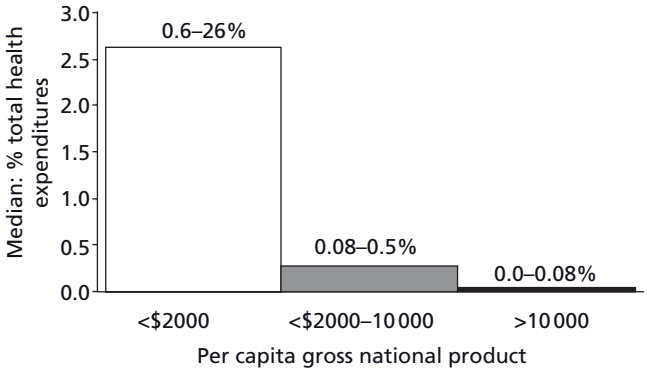

Figure 63.3 Percentage of countries' total health expenditures needed to provide minimal standardized care.

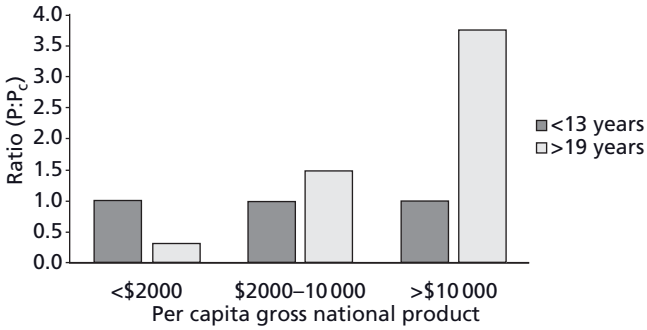

Figure 63.4 Relationship of economic capacity to the number of children with hemophilia (<13 years of age) and adult hemophilia patients (>19 years of age) [28].

comprehensive care models. The <$2000 per capita per annum group is making very slow progress in increasing levels of care.

The impact of available comprehensive care greatly affects survival. Existing data suggest that the incidence of hemophilia is constant for a number of races and ethnic groups [8]. Under these circumstances, the prevalence of hemophilia in a country might be considered a crude measurement of the overall hemophilia care because, historically, inadequate care results in premature death, shortened life expectancy, and few patients at a moment in time. When countries are grouped by GDP and examined for prevalence of hemophilia and availability of clotting factor concentrate, there is a wide gap between available care between the developed and developing world. As a result of the shortened life expectancy, the populations in the lowest level of per capita GDP have hemophilia populations that comprise primarily children (Figure 63.5). In addition, in these countries, the majority of patients will be affected by severe joint disease by their teenage years, a rare occurrence for patients living in developed countries. Although most countries report that specific laboratory diagnostic testing is used, diagnosis of hemophilia in 18–30% of the countries is still made using nonspecific tests or clinical symptoms only [21–28].

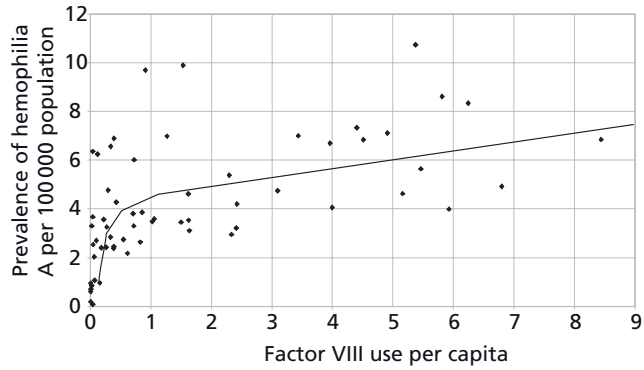

Figure 63.5 The observed prevalence of hemophilia A patients compared with the amount of factor VIII used in 60 countries reporting to the World Federation Global Survey [28]. Prevalence of hemophilia versus factor VIII use per capita (2007 data: 60 countries reported).

Developing care in countries with emerging economies

Prior to 1990, WFH development programs struggled for lack of resources. Development of the International Hemophilia Training Centers (IHTCs) by the WFH to train healthcare professionals began in 1969 and IHTC fellowships were instrumental in providing trained physicians and other health professionals to developing countries. As a result, a number of excellent centers were established [16–19,29,30]. The WFH conducted periodic workshops on hemophilia management in selected developing countries whenever funding was obtained, and the WFH Congresses, held on an annual or biannual basis, provided a forum for an interchange of ideas that stimulated interest in management of hemophilia throughout the world. A new WFH strategic plan, entitled the "Decade Plan", was published in 1992 and generated motivational energy to initiate new programs and set the goals for the WFH to make effective treatment available to patients with hemophilia globally [31].

Several new very successful programs were started between 1994 and 1997, such as the Hemophilia Center Twinning Program, which pairs emerging HTCs with well-established and experienced centers, to improve diagnosis and clinical care through coaching and training. The Hemophilia Organization Twinning Program enables emerging national hemophilia associations to develop partnerships with well-established patient organizations to share knowledge and experience in areas such as patient education, advocacy, and outreach.

Integrated, individualized country projects were soon created to target specific aspects of local hemophilia care. Intensive efforts at country-specific program development soon began. Successful programs to develop hemophilia care in countries such as Chile, Uruguay, and Venezuela provided experience and increasing optimism on achieving long-term success [32]. By working with the clinicians, healthcare teams,

hemophilia societies, and governments to put together national programs, the WFH developed sustainable progress in hemophilia care with limited resources. These country-based programs have led to significant and measurable improvement in outcomes in the management of hemophilia. They showed that the efficient use of limited resources can make a significant difference in reducing mortality and improving quality of life among people with hemophilia.

In 2003, elements of these programs were combined into an ambitious new plan, the Global Alliance for Progress (GAP) in bleeding disorders. GAP aims to introduce or expand national hemophilia care programs in up to 30 developing/emerging countries over a 10-year period and to double the number of people diagnosed with hemophilia in these countries [33]. The WFH goal is to diagnose 50 000 new people with hemophilia worldwide and improve access to care for these 50 000 and others who are currently diagnosed but untreated. Since 2003, in the first 6 years of the 10-year plan, 41 395 new patients with bleeding disorders have been diagnosed, including 31 189 with hemophilia.

One of the keys to improving care for people with bleeding disorders is building a coalition including the medical community, patient organization, and Ministry of Health. Formal agreements have been signed by WFH with the governments of Armenia, Azerbaijan, Ecuador, Egypt, Georgia, Jordan, Lebanon, Thailand, and Tunisia to establish national hemophilia programs.

In many GAP countries this has led to substantially increased levels of government support for hemophilia care. For example, the governments of Azerbaijan, Georgia, Jordan, Russia, and Thailand officially launched full National Hemophilia Care Programs and more than doubled their budgets for hemophilia in response to their engagement with WFH through GAP programs.

Outreach and diagnosis are the essential first steps in improving treatment. Since GAP began, 9675 newly identified patients with hemophilia, von Willebrand disease, and other bleeding disorders have been diagnosed and registered in the GAP countries alone.

Improving medical expertise through training of all members of the multidisciplinary healthcare team is another critical element of GAP. By 2009, WFH had provided training to over 9000 hemophilia care team members and healthcare professionals.

Increasing the availability of treatment products is also a significant turning point to bringing about adequate treatment in a country and is a cornerstone of GAP. Since 2003, GAP countries have reported to the WFH a total cumulative increase of over one billion units of clotting factor concentrates supplied in the GAP countries.

The last key piece for GAP is the patient organization. Without a strong patient group to move the project forward, advocate for improved care, and educate patients and their families, success is unlikely. Through GAP, there has been education and training of 4729 patients, family members, and patient organization board members. The increased resources and training focused on patient organizations have ensured the treatment gains will be sustainable long after the GAP project concludes.

The success of GAP has been achieved through the implementation of sustainable national comprehensive hemophilia care programs integrated into the public health system and involving government support. The WFH model for improving care is based on a series of development steps in five major areas of care: (i) level of government support; (ii) care delivery structure; (iii) level of medical expertise and diagnosis; (iv) quality and availability of treatment products; (v) strength and organization of the hemophilia association (Table 63.1). Goals and objectives are set with each country, directed toward raising the level of care by a number of realistic and sustainable steps in each area of care. Attempts are made to make these goals and objectives realistic by basing them on a careful assessment of the resources available and the chances of success in the financial and political environment of the country. These national programs can dramatically increase both life expectancy and quality for persons with hemophilia following this step-by-step approach.

Overcoming barriers to comprehensive care

Preconceptions about the cost of care has been one of the primary barriers to expanding hemophilia services to developing countries [34]. Publications concerning the high cost of hemophilia care in developed countries often generate an impression for public health planners that hemophilia care is too expensive for a country with an emerging economy, especially when concurrently faced with a number of high health priorities that affect large numbers of the population. It is not surprising, then, that the conclusion is drawn that the only option is to ignore hemophilia as a health problem.

The financing of hemophilia care is complex, however, and conclusions based on this reasoning do disservice to persons with hemophilia living in those countries because it delays low-cost elements of health care which would greatly improve survival and decrease morbidity. For example, in the developed countries the most significant cost element to the healthcare system can be attributed to the cost of clotting factor. As of 2004, health services used by patients consist of only 5% or less of the cost [35–37]. A wide variation of healthcare costs produced by individual patients has been found to be related to the severity of the hemophilia and the presence or absence of inhibitors [36]. Likewise, in any given year, as many as 24% of the patients living in a country may use no clotting factor because they do not bleed. Looking at the use of clotting factor by the approximately 17 000 patients with hemophilia A and B in the USA, of the 75% that use clotting factor, half the factor usage is for prophylaxis and immune tolerance regimens for only 15% of the population [15]. This has important implications for developing countries making decisions based on these data about undertaking national programs of

Table 63.1 Steps for developing national hemophilia care programs.

Government support	Care delivery	Medical expertise		Treatment products	Patient organization
		Laboratory diagnosis	Medical treatment		
Objectives					
To obtain government support for national hemophilia care program within the health system	To set up a national hemophilia care program (national plan defined with key treaters and NMO) To make the organization of hemophilia treatment more efficient	To provide accurate diagnosis and appropriate treatment		To obtain the best quality blood products in sufficient quantity at an affordable cost Develop and improve regulatory knowledge	To develop a strong patient organization for advocacy and education
Development steps					
No government support or interest in hemophilia care	Isolated doctor in major city works with no resources	Basic laboratory diagnostic ability	Basic medical knowledge in hematology (includes pediatricians and general practitioners)	Local production of: whole blood, plasma, fresh-frozen plasma (FFP), cryoprecipitate, freeze-dried cryoprecipitate	Organization formed by a nucleus of patients
				Combination of local production of cryo and/or FFP and some purchase of plasmaderived factor concentrates: (1) less than 0.2 IU per capita of concentrates; (2) between 0.2 and 0.5 IU; (3) between 0.5 and 1 IU; (4) between 1 and 2 IU	Organization structured, recognized/ registered with a constitution
Government recognition of main HTC as a reference center	Basic treatment is possible in hospital(s) in major city	Basic screening tests (bleeding time, platelet count, coagulation test): PT, aPTT, TT	Doctor specialized in hematology Hematologist(s) assigned to hemophilia care	Proper national tender system in place	Organization holds regular meetings with a core group of volunteers and educates patients and families in major city
Some level of government involvement in hemophilia care (e.g., hemophilia committee or task force)	Regular hematology outpatient clinic with follow-up offered	Internal quality control Factor assays	Key hematologist(s) trained in hemophilia	Examine feasibility of contract fractionation of plasma-derived concentrates	NMO patient registry

(*Continued*)

Table 63.1 *Continued*

Government support	Care delivery	Medical expertise		Treatment products	Patient organization
		Laboratory diagnosis	Medical treatment		
Limited central or regional government resources allocated for hemophilia care	Creation of a core team within hospital that forms the basis of a full hemophilia treatment center (HTC)	Participation in EQASVWD assays and inhibitor detection	Specialized hemophilia core team (hematologist, nurse, physiotherapist, orthopedist, lab technologist)	Examine feasibility of local fractionation of plasma-derived concentrates	Organizes activities including: educational services, fundraising, training, membership, volunteer recruitment, advocacy, budgeting
	Core team within hospital (HTC) has a medical patient registry and treatment guidelines/protocols				
Official government commitment to hemophilia care	Additional hemophilia treatment centers with core teams for children and/or adults in major cities	Molecular genetic detection/DNA mutation detection and carrier detection/prenatal diagnosis	Education provided to patients Home care available for patients Specialized comprehensive care team (social worker, dentist, psychologist, infectious diseases specialist, genetic counselor)	Purchase of plasma-derived concentrates (>2 IU per capita)	Outreach to other regions of the country to identify new patients Regional chapters are formed
Government contributes substantial financial support for hemophilia care	Coordinated network of designated HTCs with national treatment protocols		Education offered to general medical community	Examine feasibility of combined purchase of plasma-derived and recombinant concentrates	National organization follows a strategic plan
Hemophilia is a line item in a country's annual healthcare budget	Full comprehensive hemophilia care team is formed in the major HTC(s)				National organization is a partner in national hemophilia care program
Government is a key partner in sustainable national hemophilia care program	Basic teams formed in other areas/regions Established national medical patient registry Established sustainable national hemophilia care program				

hemophilia care [38,39]. This issue is complicated by the fact that there is wide variation between countries of the developed world in usage to determine the quantity of clotting factor most appropriate for care (Figure 63.6). The majority of developed countries use between 2 and 4 international units (IU) of factor VIII per inhabitant living in the country for their patients with hemophilia. In some countries, the quantity reaches almost 2–3 times these figures [40]. The range of

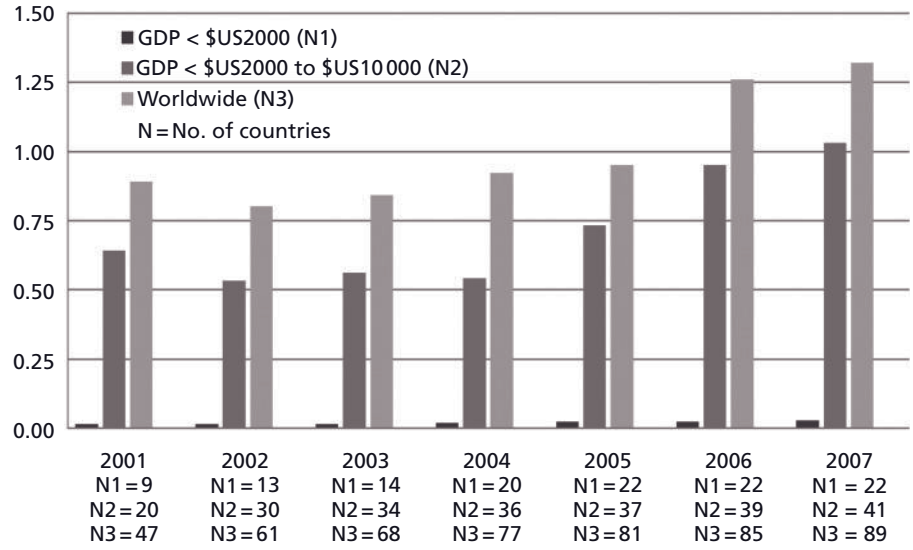

Figure 63.6 Per capita factor VIII use in emerging economies [28].

clotting factor use appears to have insignificant effect on survival (assuming that survival is reflected in the prevalence of hemophilia in the country) between the range of 1 IU per inhabitant to ≥6IU per inhabitant (Figure 63.6). The higher usage then must be accrued to other benefits, such as reducing joint disease, making possible corrective surgery for pre-existing deformities, desensitizing inhibitors, and using prophylaxis [41,42]. As noted, these benefits affect a decreasing percentage of the hemophilia population and are achieved at large increases in cost of products, rather than in funding other healthcare resources. Healthcare planners deciding to provide comprehensive care with "on-demand" therapy face an entirely different cost liability from those who plan to supply these services to include universal prophylaxis to adulthood and inhibitor desensitization therapy for all patients with inhibitors. A step-wise approach to hemophilia services, beginning with the organization of health care and blood banking services and provision of small amounts of concentrate supplemented by cryoprecipitate, can achieve much benefit; developing no program because resources do not permit everything altogether leaves much to be desired.

Conclusion

In conclusion, a wide discrepancy exists between hemophilia care availability in the developed and the developing world. Although the availability of resources is a major contributor to this discrepancy, significant improvement can be achieved by reorganization of resources and commitment to education and training. The WFH is working with many governmental agencies, healthcare providers, patient organizations, and industry to achieve this improvement to have measurable impact on the level of hemophilia care.

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References

1 Smith PS, Levine PH. The benefits of comprehensive care of hemophilia: a five-year study of outcomes. *Am J Public Health* 1984; **74**: 616–17.
2 Pool J, Hershgold E, Pappenhagen A. High-potency antihemophilic factor concentrates from cryoglobulin precipitate. *Nature* 1964; **203**: 312.
3 Levine PH, McVerry BA, Segelman AE, Cranford CM, Zimbler S. Comprehensive health care clinic for hemophiliacs. *Arch Intern Med* 1976; **136**: 792–4.
4 Smith PS, Keyes NC, Forman EN. Socioeconomic evaluation of a state-funded comprehensive hemophilia-care program. *N Engl J Med* 1982; **306**: 575–9.
5 Jones P. Hemophilia home therapy. *Homeostasis* 1992; **22**: 247–50.
6 Soucie JM, Nuss R, Evatt B, *et al.*, and the Hemophilia Surveillance System Project Investigators. Mortality among males with hemophilia: relations with source of medical care. *Blood* 2000; **96**: 437–42.
7 Soucie JM, Symons J, 4th, Evatt B, Brettler D, Huszti H, Linden J, and the Hemophilia Surveillance System Project Investigators. Home-based factor infusion therapy and hospitalization for bleeding complications among males with haemophilia. *Haemophilia* 2001; **7**: 198–206.
8 Soucie JM, Evatt BE, Jackson D, and the Hemophilia Surveillance System Project Investigators. Occurrence of hemophilia in the United States. *Am J Hematol* 1998; **59**: 288–94.
9 Manco-Johnson MJ, Riske B. Kasper CK. Advances in care of children with hemophilia. *Semin Thromb Hemost* 2003; **29**: 585–94.

10 Soucie JM, Cianfrini C, Janco RL, *et al.* Joint range of motion limitations among young males with hemophilia: prevalence and risk factors. *Blood* 2004; **103**: 2467–73.
11 Steen Carlsson K, Hojgard S, Glomstein A, *et al.* On demand vs. prophylactic treatment for severe hemophilia in Norway and Sweden: differences in treatment characteristics and outcome. *Hemophilia* 2003; **9**: 555–66.
12 Van den Berg HM, Fischer K, Van Der Bom JG. Comparing outcomes of different treatment regimens for severe hemophilia. *Haemophilia* 2003; **9**(Suppl. 1): 27–31.
13 Isarangkura P, Chuansumrit A. Developing and maintaining the hemophilia program in Thailand. *Southeast Asian J Trop Med Public Health* 1993; **24**(Suppl. 1): 61–5.
14 CDC Unpublished HSS Data, 1996–1998.
15 Evatt BL. The natural evolution of haemophilia care: developing and sustaining comprehensive care globally. *Haemophilia* 2006; **12**(Suppl. 3): 13–21.
16 National Hemophilia Survey of United States Hemophilia Treatment Centers, 2000.
17 Antunes SV. Hemophilia in the developing world: the Brazilian experience. *Haemophilia* 2002; **8**: 199–204.
18 Kim KY. Development and maintenance of hemophilia care program in Korea. *Southeast Asian J Trop Med Public Health* 1993; **24**(Suppl. 1): 52–60.
19 Fontes EM, Amorim L, Carvalho SM, Farah MB. Hemophilia care in the state of Rio de Janeiro, Brazil. *Rev Panam Salud Publica* 2003; **13**: 124–8.
20 Srivastava A, Chauansumrit A, Chandy M, Duraisamy G, Karabus C. Management of hemophilia in the developing world. *Haemophilia* 1998; **4**: 474–80.
21 WFH. *Report on the World Federation of Hemophilia's Annual Global Survey 2000*. Montreal, Canada, 2000.
22 WFH. *Report on the World Federation of Hemophilia's Annual Global Survey 2001*. Montreal, Canada, 2001.
23 WFH. *Report on the World Federation of Hemophilia's Annual Global Survey 2002*. Montreal, Canada, 2002.
24 WFH. *Report on the World Federation of Hemophilia's Annual Global Survey 2003*. Montreal, Canada, 2004.
25 WFH. *Report on the World Federation of Hemophilia's Annual Global Survey 2004*. Montreal, Canada, 2005.
26 WFH. *Report on the World Federation of Hemophilia's Annual Global Survey 2005*. Montreal, Canada, 2006.
27 WFH. *Report on the World Federation of Hemophilia's Annual Global Survey 2006*. Montreal, Canada, 2007.
28 WFH. *Report on the World Federation of Hemophilia's Annual Global Survey 2007*. Montreal, Canada, 2008.
29 Evatt BL, Robillard L. Establishing hemophilia care in developing countries: using data to overcome the barrier of pessimism. *Haemophilia* 2000; **6**: 131–4.
30 Rickard KA. Development and maintenance of hemophilia programs in developing countries. *Southeast Asian J Trop Med Public Health* 1993; **24**(Suppl. 1): 47–51.
31 Rickard KA. The International Hemophilia Training Centres of the World Federation of Hemophilia: 30-year review. *Haemophilia* 2000; **6**: 471–3.
32 Lee CA. Towards achieving global haemophilia care—World Federation of Hemophilia Programs. *Haemophilia* 1998; **4**: 463–73.
33 Skinner MW. WFH—the cornerstone of global development: 45 years of progress. *Haemophilia* 2008; **14**: 1–9.
34 Smith PS, Teutsch SM, Shaffer PA, Rolka H, Evatt B. Episodic versus prophylactic infusions for hemophilia A: a cost-effectiveness analysis. *J Pediatr* 1996; **129**: 424–31.
35 Evatt BL. Public health and international health-care development for persons with haemophilia. Operation Improvement and Operation Access. *Haemophilia* 1998; **4**: 491–7.
36 Santiago-Borrero PJ, Ortiz I, Rivera-Caragol E, Maldonado NI. Financial aspects of hemophilia care in Puerto Rico and other Latin American countries. *Haemophilia* 1999; **5**: 386–91.
37 Globe DR, Cunningham WE, Andersen R, *et al.* Hemophilia Utilization Group Study: determinant of costs of care in persons with haemophilia A. *Haemophilia* 2003; **9**: 325–31.
38 Schramm W, Royal S, Kroner B, *et al.* Clinical outcomes and resource utilization associated with haemophilia care in Europe. *Haemophilia* 2002; **8**: 33–43.
39 Srivastava A. Factor replacement therapy in hemophilia—are there models for developing countries? *Haemophilia* 2003; **9**: 391–6.
40 Srivastava A, Chuansumrit Am Chandy M, Duraiswamy G, Karagus C. Management of hemophilia in the developing world. *Haemophilia* 1998; **4**: 474–80.
41 Evatt BL. Observations from Global Survey 2001: an emerging database for progress. *Haemophilia* 2002; **8**: 153–6.
42 Miners AH, Sabin CA, Tolley KH, Lee CA. The changing patterns of factor VIII (FVIII) and factor IX (FIX) clotting factor usage in a comprehensive care centre between 1980 and 1994. *Haemophilia* 1998; **4**: 4–9.

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Notes: Page numbers in *italics* refer to figures, those in **bold** refer to tables. Coagulation factors are abbreviated to 'F' in subentries, and von Willebrand disease and von Willebrand factor to VWD and VWF, respectively.