The modern treatment of haemophilia: a narrative review

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Introduction

Haemophilia A and B are X chromosome-linked bleeding disorders that are included among the rare diseases and are caused by mutations in the genes for factor VIII (FVIII) and factor IX (FIX)[1]. Both these clotting factors are part of the intrinsic pathway of blood coagulation. Individual with haemophilia may have severe, moderate or mild forms of the diseases, defined by factor plasma levels of 1% or less, 2 to 5% and 6 to 30%, respectively. The prevalence of haemophilia A is 1 case in 5,000 male live births, while that of haemophilia B is 1 case in 30,000[2,3]. Although patients with mild haemophilia usually bleed excessively only after trauma or surgery, those with severe haemophilia experience frequent episodes of spontaneous or excessive bleeding, particularly into joints and muscles, after minor trauma[3]. The modern management of haemophilia began in the 1970s and currently includes several plasma-derived and recombinant factor VIII products[4].

This article reviews the recent history, current knowledge, the most important progress and expected improvements in haemophilia care.

The recent history of the treatment of haemophilia

In 1964 Judith Pool made the important discovery that the fraction cryoprecipitated from plasma contained large amounts of FVIII[5]. However, the modern treatment of haemophilia is considered to have started in the 1970s, with the production of lyophilized plasma concentrates of coagulation factors. This technological innovation greatly improved the quality and expectancy of life of people with haemophilia as it enabled the widespread adoption of home replacement therapy with the early control of haemorrhages and the reduction of the musculoskeletal damage typical of untreated or poorly treated patients. Specialised haemophilia centres developed programmes of comprehensive care, with the involvement of specialists such as orthopaedic surgeons, physiotherapists and dentists. Elective surgery, particularly orthopaedic operations, became possible and safe, and helped to correct or minimise the musculoskeletal abnormalities that had developed as a consequence of untreated or inadequately treated bleeding episodes into joints and muscles[6]. In parallel, primary prophylaxis was successfully pioneered in Sweden and then adopted in other countries, achieving the goal of preventing the majority of bleeding episodes and further reducing the impact of arthropathy[7]. In addition, the discovery in 1977 of the synthetic agent desmopressin provided a new, inexpensive and safe treatment for many patients with mild haemophilia A, which reduced the exposure to non-virus inactivated plasma-derived products[8].

Unfortunately, this first golden era of the treatment of haemophilia was destined to end rapidly as it became dramatically clear that plasma-derived concentrates were not only a source of life but also of death. Thus, during the first part of the 1980s haemophilia treatment entered a dark era with many shadows and very little light. This was the period of transmission of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) through contaminated coagulation factor concentrates manufactured from plasma pooled from thousands of donors[6]. Thousands of people with haemophilia died of acquired immunodeficiency syndrome (AIDS) in the 1980s and 1990s. As a consequence of the devastating sequelae of the AIDS and hepatitis epidemics, the need for safe treatment became crucial for the haemophilia community. The implementation of viral inactivation techniques for the production of plasma-derived factor concentrates, as well as the adoption of new methods to screen for viruses in donated blood (i.e., nucleic acid amplification testing [NAT]), greatly improved the safety of plasma-derived products, as shown by the fact that blood-borne transmission of hepatitis viruses or HIV has not occurred in the last 15 years[4]. However, the most important advance in this field was based on the rapid progress in DNA technology (following the cloning in 1982 and 1984 of *F8* and *F9* genes), which allowed the industrial production of recombinant FVIII (and subsequently of FIX), culminating with the publication in 1989 of the first report of clinical efficacy of this product in two patients with haemophilia A[9].

In the last two decades several improvements in the management of haemophilia patients have occurred, such that the period is usually considered a new golden era. The improvements are based on the development

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of activated recombinant factor VII (in 1996) and factor IX (in 1997), the introduction of immune tolerance programmes (in 1994), the availability of newer treatment options such as antiviral treatment against HIV (highly active antiviral therapy [HAART]) and HCV (combined therapy with α-interferon and ribavirin and liver transplantation). Table I summarises the key events of the recent history of haemophilia treatment.

The treatment of haemophilia today

As a result of the recent progress made in the field of haemophilia therapy, the life span of people with haemophilia has gradually become similar to that of males in the general population, at least in more developed countries[4]. However, with ageing, people with haemophilia develop medical and surgical conditions (e.g., cardiovascular diseases, cancers, renal disease) not previously seen in this group, which represent a new challenge for physicians working in haemophilia centres[10-12].

Another currently debated issue is that of the safety of factor concentrates. Several elements have contributed to the improved viral safety of plasma-derived products, including the adoption of quarantine for plasma units used for industrial fractionation and the introduction of NAT testing for five viruses (HIV 1-2, HBV, HCV, HAV and parvovirus B19) for most of the factor concentrates currently marketed. However, viral inactivation techniques also have a fundamental role in the viral safety of factor concentrates and have now become an integral part of their productive process. The main methods of viral inactivation, compatible with the maintenance of the biological activity of clotting factors and thus applicable to factor concentrate production, are dry heat, pasteurisation, vapour and solvent/detergent. With regards to the efficacy of these methods, it should be taken in account that while HIV is particularly sensitive to heat treatment, hepatitis viruses necessitate higher temperatures for longer periods to be inactivated and non-enveloped viruses (i.e., HAV and parvovirus B19) are not inactivated by solvent/detergent treatment. Thus, to minimise the viral infective risk, the large majority of producers have adopted two methods of inactivation, associating solvent/detergent methods with heat treatment. In addition, the viral inactivation techniques of ultrafiltration and nanofiltration, which remove smaller viruses, including non-enveloped ones, have been implemented in the last years in the preparation of FVIII and FIX concentrates. Thus, there is no doubt that plasma-derived concentrates have reached a high degree of safety in the last 20 years. However, some sentinel events should remind us that we can never lower our guard. This is the case of the documented transmission of parvovirus B19 through plasma-derived concentrates[13]. Although this event is not, in itself, relevant given the mild clinical impact of parvovius B19, it is nevertheless a signal that other clinically more important viruses, still unknown, could be resistant to currently available viral inactivation techniques and thus infect people with haemophilia through factor concentrate infusion. In addition, the recent report of post-mortem detection of variant Creutzfeldt-Jacob disease (vCJD) in a neurologically asymptomatic UK haemophiliac infused with FVIII concentrates prepared from plasma pools known to include donations from a vCJD-infected donor[14], has raised some concerns regarding the possibility of prion transmission through plasma-derived factor concentrates. Thus, with the aim of monitoring the long-term safety of replacement therapy for haemophilia, a programme named the

Table I - The recent history of haemophilia treatment.

Year	Main events
1970s: a golden era	
1970s	Lyophilised plasma-derived coagulation factor concentrates
1977	Desmopressin
1980s: many shadows, little light	
1982	Factor IX gene cloned, AIDS
1983	Early virucidal methods (dry-heating)
1984	*F8* gene cloned, HIV isolated
1985	Anti-HIV testing
1987	Safe virus-inactivated plasma-derived coagulation factor concentrates
1989	Recombinant FVIII products
1990s: a new golden era	
1994	Immune tolerance
1996	Recombinant FVIIa, HAART for HIV
1997	Recombinant FIX

European Haemophilia Safety Surveillance System (EUHASS) was started in 2008[15].

The safety of recombinant products has also increased in the last decade. Apart from the implementation of viral inactivation techniques similar to those used for plasma-derived products, much effort has been dedicated by manufacturers to the progressive removal of human and animal proteins during the production process and in the final formulation. Thus, the first generation of recombinant FVIII products used animal-derived proteins in the cell culture medium and human serum albumin was added to stabilise the final formulation. Second-generation products used human-derived proteins in the culture medium but no albumin was added to the final formulation. Finally, third-generation recombinant FVIII products, manufactured without albumin in either the culture medium or the final formulation, have now been developed[16].

Table II reports the plasma-derived and recombinant FVIII and FIX concentrates currently licensed in Italy. Plasma-derived FVIII concentrates can be classified into three subgroups depending on the type of purification procedure used: intermediate purity products obtained through conventional techniques of precipitation/adsorption, concentrates purified through ion exchange chromatography and concentrates purified through monoclonal antibodies. Recombinant FVIII products can also be divided into full-length or B-domain-deleted products, in which the B-domain is removed from the FVIII molecule without affecting its final haemostatic activity[16].

In conclusion, factor concentrates have reached excellent levels of safety in the last 15 years and nowadays the most challenging complication of the therapy of haemophilia is certainly the development of inhibitors, which renders the factor concentrate infusion ineffective thereby exposing the patients to an increased risk of morbidity and mortality[17].

Alloantibodies develop in approximately 20-30% of patients with severe haemophilia A and are much rarer in patients with severe haemophilia B in whom, however, they may be associated with

Table II - Characteristics of FVIII and FIX concentrates licensed in Italy.

Product	Manufacturer	Production characteristics	
		Purification	Viral inactivation
Plasma-derived FVIII concentrates			
Alphanate	Grifols	Heparin ligand chromatography	S/D, dry heat
Beriate	CSL Behring	Ion exchange chromatography	Pasteurisation
Emoclot D.I.	Kedrion	Ion exchange chromatography	S/D, dry heat
Fanhdi	Grifols	Heparin ligand chromatography	S/D, dry heat
Haemate P	CSL Behring	Multiple precipitation	Pasteurisation
Haemoctin	Biotest	Ion exchange chromatography	S/D, dry heat
Hemofil M	Baxter	Immunoaffinity chromatography	S/D
Immunate Stim Plus	Baxter	Ion exchange chromatography	Detergent, vapour
Recombinant FVIII concentrates			
Advate	Baxter	Immunoaffinity chromatography	S/D
Helixate NexGen	CSL Behring	Immunoaffinity chromatography	S/D, ultrafiltration
Kogenate Bayer	Bayer Healthcare	Immunoaffinity chromatography	S/D, ultrafiltration
Recombinate	Baxter	Immunoaffinity chromatography	-
Refacto	Wyeth Pharmaceuticals	Immunoaffinity chromatography	S/D
Refacto AF	Wyeth Pharmaceuticals	Immunoaffinity chromatography	S/D, nanofiltration
Plasma-derived FIX concentrates			
Aimafix	Kedrion	Anionic chromatography	S/D, dry heat
Alphanine	Grifols	Anionic chromatography	S/D, nanofiltration
Haemobionine	Biotest	Anionic and affinity chromatography	S/D, nanofiltration
Immunine Stim Plus	Baxter	Anionic chromatography	Detergent, vapour
Mononine	CSL Behring	Immunoaffinity chromatography	Sodium thiocyanate, ultrafiltration
Recombinant FIX concentrates			
Benefix	Baxter	Anionic chromatography	Ultrafiltration, nanofiltration

Legend S/D: solvent/detergent.

severe allergic reactions, such as anaphylaxis and nephritic syndrome. A number of studies have analysed conditions contributing to the development of inhibitors and have revealed the importance of genetic risk factors (e.g., ethnicity, *F8* gene mutations, major histocompatibility complex genotype, polymorphisms of immune-responses genes [interleukin-10, tumour necrosis factor-α, cytotoxic T-lymphocyte antigen-4]) and environmental risk factors (e.g., number of days exposed to FVIII, age at first exposure to FVIII concentrate, type of FVIII concentrate administered and modality of treatment). All this evidence documents that inhibitor formation in haemophilia is a complex multifactorial process[18]. Based on *in vitro* studies suggesting that the presence of von Willebrand factor in plasma-derived FVIII concentrates plays a role in decreasing FVIII immunogenicity, via epitope masking and protection of the FVIII molecule from endocytosis by antigen-presenting cells[19], the role of the type of FVIII product (i.e., plasma-derived *vs* recombinant) in the likelihood of developing inhibitors has been explored in a number of studies but with conflicting results[20-22]. In 2003, Wight and Paisley published a systematic review on the association of type of FVIII product with inhibitor formation[23]. A comparison of 13 retrospective and prospective studies on previously untreated patients (PUPs) found that patients treated with plasma-derived FVIII had a lower cumulative incidence of inhibitors than that in patients treated with recombinant FVIII (6.8% *vs* 37.5%). This difference was confirmed also for high responders inhibitors (1.4% in patients treated with plasma-derived FVIII *vs* 15.1% for patients treated with recombinant VIII). However, several methodological criticisms have been made of this review which compared trials that were very heterogeneous in terms of design (e.g., prospective/retrospective, frequency and method of inhibitor testing, duration of follow-up) and study populations (ethnicity, type of gene mutation, definition of disease severity, age at first exposure to FVIII, etc.)[24], making it impossible to draw any reasonable conclusion based on the comparison of inhibitor incidence of the different products across the studies. The recent meta-analysis by Iorio *et al.*[25] identified 420 patients how developed inhibitors among 2094 patients in 24 retrospective and prospective studies. The pooled rate of inhibitor formation was 14.3% for plasma-derived FVIII concentrates and 27.4% for recombinant FVIII products ($p < 0.001$), although the difference lost statistical significance in multivariate analysis. There were no statistical significance when high-titre inhibitors were considered (6.0% with plasma-derived FVIII *vs* 19.4% with recombinant FVIII products, $p = 0.195$). By contrast, in a more recent systematic review of data from 800 PUPs with severe haemophilia A enrolled in 25 prospective studies, we found no statistically significant difference in inhibitor rate between patients treated with plasma-derived and recombinant FVIII products (21% *vs* 27%)[26]. Similarly, the rate of high-titre inhibitors did not differ significantly between the two groups (14% with plasma-derived products *vs* 16% with recombinant FVIII products). In addition, a meta-analysis carried out by Aledort *et al.*[27], evaluating the cumulative incidence of *de novo* FVIII inhibitors in previously treated patients with severe haemophilia A, found that there was a 7- to 10-fold increased risk of new inhibitors in patients treated with B-domain-deleted recombinant FVIII compared with those treated with full-length recombinant FVIII.

Unfortunately, no randomised clinical trial is currently available to provide definite evidence on whether or not a difference in immunogenicity does indeed exist between plasma-derived and recombinant FVIII. In this regard, the results of the ongoing prospective, randomised Survey of Inhibitors in Plasma-Product Exposed Toddlers (SIPPET) are greatly awaited[28].

Conclusions

From the analysis of the data presented in this review, it can be easily concluded that the therapy of haemophilia has now reached a high degree of quality; indeed, it is probably the most efficacious and safe treatment available for a monogenic disorder.

Programmes for the future will include the production of more factor concentrate (both plasma-derived and recombinant) in order to satisfy the needs of developing countries and the development of molecules with longer half-life (such as pegylated factor concentrates) and less immunogenicity[4]. In addition, in the coming years the results of the prospective randomised trials currently underway will help us to clarify the role of secondary prophylaxis in joint status and quality of life in people with haemophilia and to identify the best immune tolerance regimen for patients with inhibitor. Finally, studies on gene therapy (the only way to cure haemophilia definitively), which were stopped because of concerns regarding the safety and efficacy of this procedure, were recently re-started in haemophilia B patients[29].

Keywords: haemophilia, therapy, hepatitis, HIV, factor concentrates.

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