Present and future challanges in the treatment of haemophilia: a clinician's perspective

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Introduction

Haemophilia A and B are rare bleeding disorders caused by mutations in the genes encoding coagulation factor VIII (FVIII) and factor IX (FIX)[1]. The prevalence of haemophilia A is 1 in 5,000 males, that of haemophilia B is 1 in 40,000[1,2]. Patients with plasma factor levels <1 IU/dL are classified as having severe haemophilia, those with levels between 1-5 IU/dL and >5 IU/dL are classified as having moderate or mild haemophilia[3]. Patients with mild haemophilia bleed excessively only in response to surgery, tooth extractions or major injuries, whereas patients with moderate haemophilia bleed excessively after relatively minor trauma and those with severe haemophilia bleed spontaneously or after trivial trauma. Severe haemophilia is epitomised by limb- or life-threatening symptoms such as haemarthrosis, soft-tissue haematoma, retroperitoneal and intracerebral haemorrhage and post-surgical bleeds. Over time, complications from recurrent joint bleeding and soft-tissue haematomas include severe arthropathy, muscle contractures and pseudotumours, leading to chronic pain and disability that often warrant orthopaedic surgery. Prophylaxis of bleeding, involving the regular infusion of products containing the deficient coagulation factor, is the mainstay of modern haemophilia care, a treatment that is evidence-based following the results of two randomised clinical trials[4,5].

Past challenges

Early significant changes in the management of these diseases took place in the 1970s, when the availability of lyophilised coagulation factors for replacement therapy permitted the development of comprehensive treatment centres and, at least in the richest countries, enabled home treatment programmes[6,7]. This decade also saw the pioneering proof-of-concept and initiation in Sweden of prophylaxis regimens[8], as well as the development of desmopressin (DDAVP) for the treatment of mild haemophilia A and some types of von Willebrand disease (VWD)[9].

The 1980s were characterised by many shadows but also by some lights. These were the years of the widespread occurrence in patients with haemophilia of AIDS and hepatitis, but these scourges fostered research that led to the cloning of factor VIII (FVIII) and factor IX (FIX) genes, the basis for the production of coagulation factors by recombinant DNA technology[10]. Progress in viral inactivation methods also made plasma factor products pathogen-free and much safer, so that since the late 1980s/early 1990s, no clinically significant pathogens have been transmitted to patients by antihaemophilic products[1,2].

The 1990s heralded a new favorable era in haemophilia treatment, which continues now into the third millennium. Recombinant FVIII (rFVIII) and recombinant FIX (rFIX) were industrially produced, investigated clinically and licensed during this decade[10]. Importantly, those patients who previously had no effective treatment of actual bleeding episodes due to the development of inhibitors to infused FVIII, and more seldom to FIX, witnessed substantial therapeutic progress through the development and availability of products such as prothrombin complex concentrates (PCC), FVIII inhibitor bypassing activity (FEIBA) and recombinant activated factor VII (rFVIIa). These therapeutic weapons permitted bleeding episodes to be treated almost as effectively as in patients without inhibitory alloantibodies[11,12]. The 1990s also witnessed the introduction of immune tolerance induction that permanently eradicates inhibitors (particularly those to FVIII) in at least 60-70% of patients affected by this complication[13]. Another advance was the availability of drugs (highly active antiretroviral therapy for HIV; interferon and ribavirin for hepatitis) which dramatically changed clinical outcomes in those haemophiliacs who had acquired these blood-borne diseases before the advent of safer plasma-derived products in the late 1980s - early 1990s[14-16].

As a direct result of these developments, life expectancy for patients with haemophilia in many countries has currently approached that of the general population[15,16]. Thus, among the most frequent monogenic diseases, patients with haemophilia are much better off in terms of life quality and expectancy than those with cystic fibrosis, thalassemia major or muscular dystrophy.

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Haemophilia in the Third Millennium

On the basis of the progress witnessed over the past 40 years, it is not surprising that the motto of haemophilia management in the third millennium is "building on strength". However, considerable scope remains for further advances, the followings being the main issues of current treatment.

Greater and wider factor availability

Both plasma-derived (pd) and recombinant (r) factor products are efficacious and safe, as recently confirmed by means of regular monitoring of more than 30,000 patients with inherited bleeding disorders via the European Haemophilia Safety Surveillance (EUHASS) system. At present, however, at least two-thirds of the world's population with haemophilia (more than 400,000 people) remain without access to any coagulation factor therapy. At the heart of this issue is not only availability but also cost. More than 20 years after the introduction of recombinant factors and the forementioned modern treatment arsenal, the cost of replacement therapy in most countries has not decreased, with the exception of the UK where a well-balanced tender system has decreased the cost of recombinant products by 30-40% without jeopardizing the quality of treatment and with the agreement of most stakeholders. Australia has developed a similar system for cost saving. It is hoped that very large countries such as China and India, which have a capacity for high technology, will choose to develop bio-similar recombinant coagulation factors and introduce them, not only in their countries but also in the general marketplace.

Less inhibitors in previously untreated patients (PUPs)

Current haemophilia therapy is compromised by the immunogenic potential of therapeutic factor products, as shown by the fact that the cumulative incidence of inhibitors in the overall population of patients with haemophilia A is estimated to be approximately 30-35%[17]. The highest risk of inhibitor development is in children aged 0-4 years who are previously untreated (or minimally treated) with factor replacement therapy.

A number of patient and environment-related factors are associated with the risk of developing FVIII inhibitors in PUPs with haemophilia A[12]. The present focus is on whether or not there is a different immunogenic potential between recombinant (rFVIII) and plasma-derived FVIII (pdFVIII) used for replacement therapy. Post-translational modifications (e.g. glycosylation) occur more frequently in highly engineered rFVIII than in native pd FVIII[18,19]. For the highly purified FVIII products, it is also possible that some important immunosuppressive molecules present in plasma-derived products (e.g. TGFβ) may be missing. The presence of large amount of von Willebrand factor (VWF) in the majority of pd FVIII products is thought to decrease the likelihood of inhibitor occurrence.

A systematic review reporting the incidence of inhibitors in PUPs with haemophilia A suggested that the immunogenicity of rFVIII is more than twice that of pd FVIII (27.0 *vs* 10.8%)[17]. A subsequent meta-analysis involved a total of 2,094 patients, 1,965 of whom were treated with pd-FVIII and 887 with rFVIII, and found that the risk of inhibitor with rFVIII was approximately twice than that of with pd FVIII (27.4 *vs* 14.3%)[20]. However, the statistical significance of this difference was lost when variables such as study design, study period and inhibitor testing frequency were taken into account. As with any meta-analysis, results are dependent on the highly heterogeneous quality of the studies included and these findings must be regarded as hypothesis-generating rather than conclusive for therapeutic choices. Regarding the role of the FVIII source on inhibitor incidence in PUPs with haemophilia A, there is, at the moment, uncertainty regarding differences between available products from different sources, providing justification to randomise patients to competing therapies. This was the rationale for the ongoing randomised SIPPET (Study on Inhibitors in Plasma-Product Exposed Toddlers)[21], which is evaluating the hypothesis that pdFVIII/VWF products are less immunogenic than rFVIII products. In order to provide information valuable for all FVIII products and not just for a single entity, patients with severe haemophilia enrolled in the SIPPET study are randomised to receive, throughout the study, only a single product from each of the two classes of FVIII (VWF-containing pd FVIII or rFVIII products). This international independent study, conducted in 4 continents, started to enroll PUPs in 2010 and has the goal of collecting data from 300 patients. Although the final results are still some time away owing to the rarity of these patients and competition with studies sponsored by the industry, it is hoped that SIPPET will ultimately provide unbiased answers to this important clinical dilemma.

Novel information on this issue has recently come to light since the publication of the results of RODIN, an observational cohort study on the development of FVIII inhibitors in 574 PUPs with severe haemophilia A from Europe, Canada and Israel[22]. The main results indicated no difference in the cumulative incidence of inhibitors in patients treated with pdFVIII or rFVIII products. Moreover, in the frame of an unplanned post-hoc analysis, the risk of inhibitor development was 60% higher in patients treated with second generation, full-length rFVIII compared with those receiving third generation, full-length rFVIII[22]. RODIN is the largest

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study conducted so far in PUPs with severe haemophilia, and its results are important. Yet this study has a number of methodological limitations. The finding of a similar rate of inhibitors in patients treated with pdFVIII and rFVIII is rendered uncertain by the small absolute number of those who received pdFVIII, and by the fact that pdFVIII products containing little or no VWF were conglomerated in the analysis together with products rich in VWF. Owing to the observational design of RODIN, there was no random assignment of patients to products of different source and generation, nor were attempts made to account for the varied propensity of choosing one product rather than another. Lack of accounting for differential patient assignment to products may have introduced selection bias, thus affecting the final results. Regarding the reported increased risk of inhibitors for patients who received second generation full-length rFVIII , there is no straightforward biologically plausible explanation for the difference *versus* the third generation rFVIII product arbitrarily used as the only reference to estimate the risk of inhibitor development. For instance, the prospective EPIC study (http://www.clinicaltrials.gov, #NCT01376700), based upon the exclusive use in PUPs of the latter product, was recently stopped because of an unexpectedly high rate of inhibitor development. Finally, these and other results of RODIN should be taken in the context of all the available evidence on inhibitor development in PUPs. A very wide range of cumulative incidences (from 0% to as much as 52%, averaging 30%) was reported in previous smaller studies and the inhibitor rate for all FVIII products evaluated in RODIN was within this wide range. Other comments on the limitations of RODIN are reported by Kessler and Iorio[23]. All in all, RODIN does not constitute final evidence of equal inhibitor risk between pd and rFVIII, nor of higher risk for the second generation full-length rFVIII. Hence, its results do not impinge upon the views that all the currently available FVIII products are similarly safe and effective. Only a randomised, head-to-head study such as SIPPET can truly determine the comparative incidence of inhibitors associated with different sources of FVIII products.

Development of inhibitors in previously treated patients

Is switching from one FVIII product to another a risk factor for inhibitor development? The earliest data on the incidence of inhibitor development after switching therapy derive from two surveillance studies conducted in Canada[24,25]. The first, published in 1998, reported a 2-3% incidence of inhibitors over a 2-year follow-up period in 478 inhibitor-free patients switched from pd FVIII to a first-generation rFVIII[24]; this incidence of new inhibitors being stated by the authors to be similar to that observed before switching. A decade later, the same group reported that no de novo inhibitor developed during 2-years of follow-up in 274 patients with haemophilia A after being switched to a second-generation rFVIII of the same commercial brand as their previous first-generation product[25].

Furthermore, a meta-analysis of prospective clinical studies was conducted to test the hypothesis that the incidence of de novo inhibitors differs between PTPs who receive full-length rFVIII (FL-rFVIII) and those who receive B-domain deleted recombinant FVIII (BDD-rFVIII)[26]. This is an important clinical question because in the near future it is expected that most FVIII products will be B-domainless. Moreover, if gene transfer therapy in haemophilia A ever reaches the clinical stage it will almost certainly be based upon the transfection with a B-domain deleted FVIII gene. Overall, the incidence of inhibitors was 0.83 per 1,000 patient/years (95% CI: 0.46-1.52) for pooled FL-rFVIII and BDD-rFVIII; 0.42 (0.19-0.93) for pooled FL-rFVIII; and more than 6 times higher (2.61, 1.21-5.53) for pooled BDD-rFVIII. Once again, however, the extension of the results of this meta-analysis to a general conclusion is limited by the quality of the studies included. The absolute number of patients with inhibitors was small (35 in total) and the confidence interval for the pooled BDD-rFVIII results was very large, introducing the possibility of type 1 error. The main message that stems from this meta-analysis is that the incidence of de novo inhibitors in PTPs receiving any rFVIII concentrate (either full length or B-domain deleted) is very low, as substantiated by the narrow confidence interval and by similar results reported in other analyses[27].

Longer-acting rFVIII and rFIX

Current replacement therapy in haemophilia is relatively inconvenient because the most widely used prophylaxis regimens involve repeated intravenous infusions, generally 2-3 times weekly or even daily in some instances[28], owing to the short half-life of 10-12 hours for the available FVIII products (slightly longer for FIX). For this reason, approaches to prolong factor half-life in the circulation have been developed to improve factor replacement therapy for patients with haemophilia. The potential benefits of longer acting factors include extended protection from bleeding, reduced infusion frequency and hence less need to apply a central catheter for venous access with its associated problems[28]. The main approaches under current investigation involve hydrophilic polymer conjugation (e.g. PEGylation) and variant protein generation (e.g. fusion protein strategy)[28-31].

Chemical conjugation has been achieved mainly by means of polyethylene glycol conjugation (Pegylation), in which linear *vs* branched PEGs and random *vs* site-

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specific PEG attachments to coagulation proteins have been attempted. A phase I trial in 26 PTPs with severe haemophilia has found that the pegylated BDD FVIII was well tolerated and that the mean half-life was 19 hours, i.e., a 1.6 fold prolongation in comparison with standard BDD FVIII[32]. Site-directed glycoPEGylation appears to have an even greater place in terms of improving the pharmacokinetic properties of rFIX. In the first human trial conducted in 16 PTPs with haemophilia B, the half-life of pegylated recombinant FIX at the same dose level as their previous standard FIX product was five times longer (93 h *vs* 18 h)[33]. This represents a substantial difference and suggests that such a formulation of FIX could be administered at intervals of once every 10-15 days.

Fusion protein is another promising strategy to prolong the half-life of rFVIII and rFIX and involves genetic engineering of fusion constructs of coagulation proteins with either albumin or immunoglobulin. Studies using a FIX/monomeric Fc immunoglobulin fusion protein have shown that the modified FIX protein offered a 3- to 4-fold extension of half-life[34]. For the corresponding FVIII/Fc fusion protein, the mean half-life was 1.6 times longer than that of standard FVIII, thus much shorter than that of the corresponding FIX fusion protein[35]. Finally, a recombinant FIX obtained by the fusion of rFIX with recombinant human albumin was characterised in 25 PTPs with haemophilia B, showing that the mean half life of the fusion protein was at least five times longer than that of standard FIX[36].

Despite these remarkable preliminary results for the strategies intended to prolong the half-life of recombinant coagulation factors, a number of unresolved questions remain and await for clarification to come from the completion and publication of the ongoing clinical studies[30]. What is the potential for immunogenicity of these new proteins endowed with such an extensive manipulation of the molecule? Will prolongation of the FVIII half-life prove to be as significant and attainable as that for FIX? What degree of half-life extension will be required to justify a change in clinical practice, bearing in mind the potential risks and definitely higher costs of such a change? Whereas moving from thrice- to twice-weekly prophylactic intravenous infusions would change only moderately the patients' quality of life, a shift to weekly or fortnightly infusions for the entire haemophilia population would represent substantial progress.

Needless to say, persons with haemophilia, although satisfied with the current status of their treatment, expect to be cured from the disease. Although some preliminary results obtained with gene replacement therapy in patients with severe haemophilia B are quite promising[37], gene transfer is still relativly far from being implemented on a large scale, particularly for haemophilia A.

Keywords: haemophilia, factor VIII, factor IX, inhibitors, recombinant technology.

Conflicts of interest disclosure

Pier Mannuccio Mannucci, as member of an independent committee for the Bayer Awards, had received honoraria. He has also received honoraria for participating as speaker at educational meetings organised by Biotest, Bayer, Grifols, Kedrion Biopharma and Novo Nordisk.
Massimo Franchini has no conflicts of interest.

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