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Coagulation factor concentrates: past, present, and future

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Clotting factor transfusions are vital for people with diseases such as haemophilia. In the 1970s and 1980s, transfusions with pooled plasma led to a devastatingly high number of recipients becoming infected with blood-borne pathogens such as HIV and hepatitis C. This epidemic triggered the development of virus-free factor concentrates through a combination of improved donor selection and screening, effective virucidal technologies, and recombinant protein expression biotechnology. There is now a wide range of recombinant factor concentrates, and an impressive safety record with respect to pathogen transmission. However, remaining therapeutic challenges include the potential threat of transmission of prions and other pathogens, the formation of inhibitory alloantibodies, and the international disparity that exists in product availability due to differences in licensure status as well as prohibitively high costs. In the future, it is likely that bioengineered recombinant proteins that have been modified to enhance pharmacokinetic properties or reduce immunogenicity, or both, will be used increasingly in clinical practice.

Introduction

Inherited clotting factor deficiencies are rare, with prevalences between 1 in 10 000 to 1 in 10 000 000. Acquired factor deficiency states, however, are common, and are seen in many pathological conditions. Fresh-frozen plasma (FFP) and cryoprecipitate have traditionally been the mainstays of treatment for inherited coagulopathies. Their use is falling because of concerns over blood-borne pathogen transmission, but the products are still useful when no specific fractionated product is available (eg, factor V deficiency), and in complex acquired coagulopathies characterised by deficiencies of multiple clotting factors (eg, in bleeding from disseminated intravascular coagulation [DIC] or liver disease). Cryoprecipitate is enriched in cryoprotein factor VIII (FVIII), Von Willebrand factor (VWF), FXIII, fibronectin, and fibrinogen. Where available, a pathogen-inactivated form of FFP is recommended.¹ However, no similarly treated cryoprecipitate products are available.

Plasma that is fractionated to generate clotting factor concentrates falls into two categories: recovered plasma from whole blood donations (generally uncompensated) through licensed blood banks, and source plasma collected by apheresis, generally using paid donors. The

range and specifics of viral testing of individual donors varies internationally, especially since many serological tests are gradually being replaced by direct viral genomic detection using nucleic acid testing. Typically, frozen source plasma is first fractionated into cryoprecipitate by slow thawing. The “cryopaste” can then be used to manufacture FVIII concentrates by, for example, precipitation, gel permeation, or ion exchange chromatography, or by affinity chromatography using immobilised monoclonal antibodies. Cryo-poor plasma (plasma from which the cryoprecipitate has been removed) can be further processed to prothrombin complex or other single factor concentrates, or both.

During the late 1970s and early 1980s, the pooling of plasma from 2000 or more donors in clotting factor concentrates (with no virucidal steps) led to an international disaster, in which a large number of haemophiliac patients became infected by blood-borne viruses, particularly HIV and hepatitis C. An estimated 9300 haemophiliacs in the USA (almost half the total number) were infected by HIV,² and the proportion of those infected by hepatitis C was probably closer to 80%. As a result of this, virucidal methods—most commonly a combination of solvent detergent exposure, nanofiltration, or exposure to heat either as a lyophilised product (“dry heat”) or in the aqueous phase (“pasteurisation”)—were introduced to inactivate infectious particles. However, despite the excellent safety record of these techniques in preventing transmission of lipid-enveloped viruses since the mid 1980s, non lipid-enveloped pathogens such as parvovirus B19 could survive the process and be transmissible.^{3,4} In addition, concerns remain regarding the possible transmission of prions by clotting factors, although no documented cases have been reported.^{5,6}

Internationally, there is considerable variation in the availability and licensed indications for many products. Both plasma-derived and recombinant FVIII and FIX have been widely available for several years. Other

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Search strategy and selection criteria

We undertook a systematic search of articles published before September, 2006, in English using the PubMed database. Search terms included keywords such as “factor VIII”, “von Willebrand factor”, “prothrombin complex”, and “activated protein C”. These categorical search terms were cross-referenced with other keywords, including “inherited deficiency”, “acquired deficiency”, “plasma derived”, “recombinant”, “pathogen”, “treatment”, “thrombogenicity”, and “clinical trial”. To limit the number of references, review articles, and recent articles were given preference.

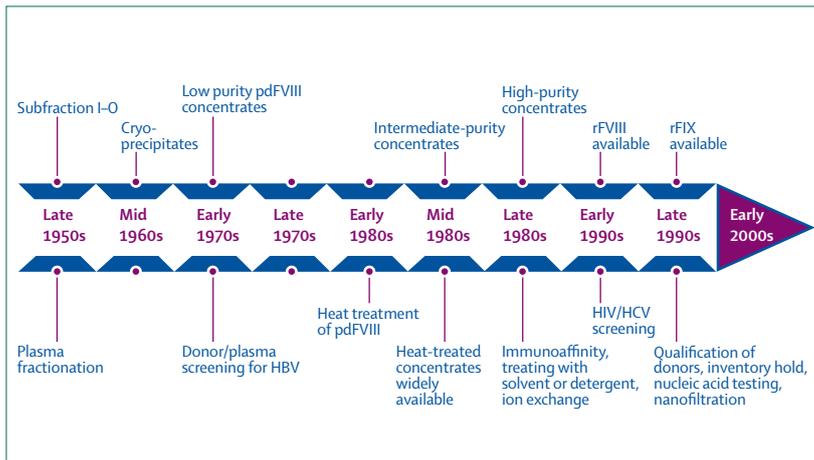


Figure: Historical scheme of FVIII and FIX concentrate development

single-factor concentrates fractionated from pooled donor plasma such as fibrinogen, VWF, FVII, FXI, FXIII, and protein C are marketed in some countries. Recombinant forms of several other factors, including FVIIa, FXIII, antithrombin, and activated protein C are either available or are currently undergoing pre-licensing clinical trials. The World Federation of Hemophilia's Registry of Clotting Factor Concentrates⁷ has a more exhaustive listing, details of plasma source, fractionation and virucidal methodologies, and additional pertinent information about individual products. This article will review the range of contemporary coagulation factor products and their uses in inherited and acquired disorders.

Clotting factor concentrates in inherited and acquired bleeding disorders

The treatment goal in these disorders is usually to replace the missing coagulation factor from external sources.⁸ The conventional treatment approach is episodic, in which the missing factor concentrate is administered as soon as possible after the onset of bleeding. Occasionally, a prophylactic approach is used, in which the coagulation factor is given according to a regularly prescribed schedule to prevent bleeding.

In primary prophylaxis, the concentrate is given from an early age to prevent expected complications (such as repeated haemarthroses in haemophilia, or intracerebral haemorrhage in FXIII deficiency), whereas secondary prophylaxis is begun after such events occur, to prevent recurrence.⁹ The rationale for primary prophylaxis in haemophilia has recently been validated in a prospective randomised controlled trial in the USA.¹⁰ A dose-escalated prophylaxis regimen for haemophilia (increasing the frequency from once weekly until breakthrough bleeds are controlled) has been investigated.¹¹ The once a week regimen resulted in fewer bleeds (and fewer "target joints") than historical controls.

As a general rule, one unit of FVII, FVIII, FXI, FXIII, or VWF per kg body weight will raise the respective factor activity in the recipient's plasma by 1.5–2.0 IU/dL (1.5–2.0%), whereas the expected recovery for FIX is 0.7–1.4 IU/dL/U/kg infused.

As transmission of blood-borne pathogens has decreased, the development of inhibitory antibodies to the transfused clotting factor has become the most serious treatment complication, with a cumulative incidence up to 30% in previously untreated patients with severe haemophilia A with first-generation^{12–14} and second-generation rFVIII.¹⁵ Although the risk of developing inhibitory antibodies is partly determined by the specific underlying mutation and severity of the deficiency, concerns remain about the relative immunogenicity of various types of concentrate. In Europe, the development of inhibitory antibodies in low risk patients after introduction of modified plasma-derived FVIII (pdFVIII) concentrates have been reported in two well documented case series reports.^{16,17} A 2006 retrospective (albeit non-randomised) study from France continues to raise the question that pdFVIII might be less likely to lead to inhibitor development than rFVIII.¹⁸

FVIII concentrates

Satisfactory management of haemophilia only became possible with the development of plasma-derived clotting factors concentrates in the late 1960s.^{19,20} In the subsequent two decades, FVIII and FIX concentrates were produced exclusively from human plasma. Plasma from multiple donors was pooled, but this practice was a major contributor to the transmission of blood-borne infectious agents such as hepatitis B, hepatitis C^{21–23} and HIV.^{24–26} The subsequent evolution of coagulation-factor replacement therapy focused on maximising viral safety through the widespread implementation of donor selection and screening tests and of chromatographic purification and viral inactivation steps (figure).

In the early 1980s, the cloning and sequencing of FVIII initiated the development of rFVIII.^{27,28} Human rFVIII can only be produced using mammalian cell-culture systems (Chinese hamster ovary cells or baby hamster kidney cells) due to the complex glycosylation and other post-translational modifications required for its full cofactor activity. Scale-up of production and purification processes led to the commercial production of the first full-sequence length rFVIII products—a major biotechnological achievement. In addition, culture media, which used to contain human and animal-derived proteins, now contain chemically synthesised or genetically engineered molecules instead. The purification process removes impurities derived from the medium and cultured cells, and concentrates the rFVIII molecule through various chromatographic steps. All currently available rFVIII products are purified using immunoaffinity chromatography using a murine monoclonal antibody directed against human FVIII.

Although viral transmission has never been recorded with any rFVIII product, a theoretical risk of transmitting a human-derived infectious agent still remains in the first-generation products, in which human and animal proteins were not completely eliminated from the production process. In addition, emerging non-viral pathogens such as the prion responsible for variant Creutzfeldt-Jakob disease (vCJD) must be considered, and reducing the risks of pathogen transmission continues to be a high priority for the haemophilia community.^{6,29}

Recombinant FVIII products have excellent haemostatic efficacy in both previously untreated and treated haemophilia A patients. Since the manufacture of rFVIII is not limited by plasma availability, the improved supply of FVIII in developed countries has contributed to increased application of prophylactic treatment regimens and subsequent improvement of functional outcomes.^{9,30}

FIX concentrates and prothrombin complex concentrates FFP or plasma derivatives (prothrombin complex concentrates [PCCs]; otherwise known as intermediate purity FIX concentrates) were used as the source of FIX in haemophilia B. Prepared either by Cohn fractionation or calcium adsorption of plasma, PCCs were first introduced in the early 1970s (figure).^{31,32} These agents are enriched in prothrombin and factors VII, IX, and X, and also contain trace amounts of factors VIII, VIIa and IXa. However, the specific content of each clotting factor, particularly FVII, varies by concentrate.^{33,34} The anticoagulant vitamin K-dependent factors protein C and protein S are also present at variable concentrations.

Thrombotic events, including venous thromboembolism and DIC,³⁵⁻³⁷ as well as microvascular thrombosis and myocardial infarction³⁸ have been reported with the use of PCCs. These complications seem to occur especially, but not exclusively, with the use of frequent or high dose (>200 U/kg/day) administration. Particular concern for a raised risk of thrombotic complications and DIC has been expressed with regard to patients with severe liver disease, possibly because of their failure to adequately clear activated clotting factors from the circulation.^{34,36,39} Consequently, measures to reduce the thrombogenicity of these concentrates were taken by the manufacturers that included the addition of heparin, and antithrombin or protein C, or both.

About 15 PCCs are marketed worldwide.⁷ Vial potency labeling and dosing recommendations for PCCs are based on IUs of FIX. One IU of FIX corresponds to the activity of FIX in 1 ml of fresh normal human plasma.

The use of PCCs in haemophilia B fell after the introduction of high purity pdFIX (and subsequently rFIX) products in the 1990s. By contrast with PCCs, infusion of these high-purity FIX products did not lead to any significant activation of the coagulation system,⁴⁰ confirming that a component other than FIX is responsible for the thrombogenicity of PCCs in

haemophilia B. Evidence suggests that it could be excess prothrombin, rather than the content of activated factors VII, IX, or X in these concentrates that is primarily responsible for thromboembolic complications.^{41,42}

PCCs remain a useful treatment for other inherited and acquired coagulation factor deficiency states, for example, in the prevention or treatment of bleeding in inherited factor X or II (prothrombin) deficiency.⁴³ The use of PCCs as an alternative to FFP has also been reported in some complex acquired bleeding disorders, including dilutional coagulopathy from massive transfusion,⁴⁴ bleeding after cardio-pulmonary bypass surgery,⁴⁵ and the coagulopathy of acute fulminant and chronic liver failure.^{46,47} However, there are few reports of all these situations, and neither the risk-benefit profiles nor the optimal dosing regimens have been established.

The human FIX gene was cloned in the early 1980s, which led to the expression of human rFIX in CHO cells.^{48,49} Recombinant FIX (Nonacog alfa, Wyeth, PA, USA) is structurally and functionally similar to pdFIX, although minor differences in the post-translational sulfation and phosphorylation of rFIX have been associated with about 30% lower *in vivo* recovery, especially in children ≤15 years of age.⁵⁰ International clinical trials have demonstrated the efficacy and safety of rFIX for the treatment of haemorrhages as well as in prophylactic and surgical settings in previously treated patients (PTPs) and in previously untreated patients (PUPs) with haemophilia B.⁵⁰

The bypassing agents: FEIBA and recombinant factor VIIa (rFVIIa)

The need for therapies to control bleeding in haemophilia patients affected by high titre inhibitors to FVIII or FIX—that is, to “bypass” the FVIII/IX complex in coagulation—led to a number of early clinical trials exploring the efficacy and safety of PCCs⁵¹⁻⁵³ (table 1). In the 1970s, the first activated PCCs were developed. These

	Study groups	Doses (n)	Total patients (n)	Response rate* (%)
1980 ⁵³	Konyne	1	157	47%
	Proplex	1		53%
	Placebo	1		25%
1981 ⁵²	FEIBA‡	1 or 2	150	64%†
	Prothrombex	1 or 2		52%
1983 ⁵¹	Autoplex‡	1	82	52%
	Proplex	1		56%
2007 ⁵⁴	FEIBA-VH‡	1	48	81%
	rFVIIa	2		79%

*Generally assessed by subjective judgment at 6 h post-infusion. †p<0.05.

‡Activated PCC.

Table 1: Randomised clinical trials of bypassing agents (prothrombin complex concentrates (PCC), activated PCCs, and rFVIIa) in the treatment of mild to moderate bleeds in haemophilia complicated by an inhibitor

products were manipulated *ex vivo* to increase the content of activated clotting factors, especially FVIIa. At present, only one product, FEIBA (an acronym for Factor Eight Inhibitor Bypassing Activity) VH anti-inhibitor coagulant complex (Baxter Bioscience, Vienna, Austria) is available for this indication.

The vial potency labelling is in arbitrary units of FVIII inhibitor bypassing units, where one unit of FEIBA-VH shortens the activated partial thromboplastin time (aPTT) of high-titre FVIII reference plasma to 50% of the blank value. The drug's mechanism of action is now believed to be dependent on its content of prothrombin and FXa.⁵⁵ Empirically, FEIBA is administered at doses of 50–75 units/kg every 8–12 h, with a recommended maximum daily dose of 200 units/kg. Three early-1980s prospective randomised clinical trials on the early treatment of acute haemarthrosis established the efficacy and safety of PCCs and factor VIII inhibitor bypassing fraction.^{52–54}

Notably however, the response rate, judged subjectively by joint pain resolution, was only 50–60% at 6 h after the first infusion (with significantly higher rates of response for the drug compared with a non-activated PCC), compared to a placebo response rate of 25% (see table 1). These response rates at 6 h are significantly lower than would be expected when using FVIII to treat acute haemarthrosis in haemophilia A uncomplicated by an inhibitor. With repeated dosing over longer periods however, the efficacy rate for FEIBA in the management of acute bleeding events is substantially higher, generally more than 85%.^{56,57}

While partial correction of the prolonged aPTT is typical in haemophilia patients treated with FEIBA, this parameter does not represent a clinically useful laboratory monitoring strategy. Two studies in the past few years have assessed alternatives, including thromboelastography and thrombin generation profiles (“endogenous thrombin potential”) in whole blood and plasma, respectively.^{58,59}

Recombinant factor VIIa (rFVIIa; Eptacog alfa [activated], Novo Nordisk, Bagsvaerd, Denmark) is almost structurally identical to native FVIIa. This agent is widely licensed for the management of bleeding in haemophilia A or B complicated by inhibitory antibodies (at doses of 90–120 µg/kg) and for inherited FVII deficiency (at a dose of 15–30 µg/kg), and in Europe for bleeding in Glanzmann's thrombasthenia with refractoriness to platelet transfusions due to antibodies to GP IIb-IIIa or HLA. In the haemophilias, high dose rFVIIa is believed to act by producing a “thrombin burst” on the surface of activated platelets by proteolytic activation of factors IX and X (and ultimately prothrombin) in the absence of tissue factor.⁶⁰

Although some data have suggested increased efficacy with even higher doses of rFVIIa (usually 270 µg/kg) in haemophilia-related bleeding,⁶¹ supportive data from prospective randomised clinical trials have thus far only

shown equivalence.⁶² Like FEIBA, the haemostatic efficacy rates for rFVIIa vary depending when after administration it is judged; indeed, a recent multinational randomised cross-over clinical trial (FENOC⁵⁴) demonstrated equivalence of a 85 U/kg dose of factor VIII inhibitor bypassing fraction and two 105 µg/kg doses of rFVIIa. Response to both was judged to be “effective” in about 80% of cases at 6 h (table 1). Regardless of the indication, administration of rFVIIa invariably results in shortening of the prothrombin time, although this does not correlate with haemostatic efficacy. As with factor VIII inhibitor bypassing fraction, a validated method for monitoring rFVIIa is an area of active investigation.⁶³ The precise indications for the use of bypassing agents in haemophilia, as well as their relative merits and drawbacks have been reviewed elsewhere.^{64–66}

Von Willebrand factor concentrates

Until 2001, cryoprecipitate was the therapeutic mainstay for bleeding in VWD. In 2001, because of concerns for the potential transmission of blood-borne pathogens, the use of pdFVIII products enriched in VWF was recommended for the treatment of bleeding or prophylaxis before surgery in certain sub-types of VWD.⁶⁷ Factor concentrates are generally recommended for most patients with type 2 variants (qualitative defects) of VWD, and both severe type 1 and type 3 variants (partial quantitative and severe quantitative deficiencies of VWF, respectively). Most remaining patients with milder variants of type 1 VWD respond well to intravenous, subcutaneous, or intranasal desmopressin (1-deamino-8-D-arginine vasopressin; DDAVP).^{68,69}

Although FVIII synthesis is not defective in VWD, its half-life is severely reduced when VWF, its natural carrier and stabiliser to which it is non-covalently bound in plasma, is deficient. Satisfactory haemostasis in VWD is dependent on achieving adequate plasma levels of both VWF (mediating primary haemostasis) and FVIII (responsible for fibrin formation in secondary haemostasis). As a rule, haemostasis is satisfactory when the ristocetin cofactor activity (VWF:RCo)—a measure of VWF activity—is more than 0.6 IU/ml (60% of normal). In the absence of an rVWF concentrate, most products are intermediate purity pdFVIII concentrates that also contain VWF, and thus may be used in the treatment of either haemophilia A or VWD.^{70–72} The only exception is Wilfactin (LFB, Lille, France), a plasma-derived product that is considered to be a highly purified VWF-containing concentrate, although it is only available in a few European markets. Although administration of this product to a patient with severe type 1 or type 3 VWD quickly corrects the plasma deficiency of VWF, there is a delay of 6–12 h before endogenous FVIII activity is restored to haemostatic levels. Thus, protocols in which highly purified VWF is used to control active bleeding generally recommend a single supplemental dose of high purity FVIII concentrate at the onset of therapy.⁷³

	Manufacturer	Approximate VWF:Rco/FVIII:c ratio	Half life of VWF:RCo	VWF:RCo recovery (IU/dL/IU/kg injected)	Viral inactivation method	Licence status
Alphanate ⁷¹	Grifols (Barcelona, Spain)	0.6:1	7.1 h	2.5	SD, then dry heat at 80°C for 72 h	Licensed in Italy, the UK, and the USA
Wilate ⁷²	Octapharma (Lachen, Switzerland)	1.0:1	17.1 h	1.5	SD, then dry heat at 100°C for 72 h	Limited European licensure
Humate-P/Haemate-P ⁷²	ZLB Behring (Marburg, Germany)	2.4:1	10.3 h	1.9	Pasteurisation at 60°C for 10 h	Licensed in North America and Europe
Wilfactin (formerly Facteur Willebrand-LFB) ⁷³	LFB (Lille, France)	>10:1	12 h	2.1	SD, then 35 nm nanofiltration, followed by dry heat at 80°C for 72 h	Limited European licensure

SD=treated with solvent detergent.

Table 2: Some concentrates for the treatment of Von Willebrand disease

Table 2 shows that the ratio of VWF:RCo to FVIII activity (FVIII:c) is extremely variable among the available products. Qualitatively, there is also significant variation in the degree of preservation of the larger VWF multimers required for platelet adhesion to sub-endothelial collagen, although the clinical relevance of this finding is unknown.⁷⁴

Fibrinogen concentrates

Afibrinogenaeic patients can develop life-threatening bleeding symptoms that can usually be controlled by fibrinogen replacement or cryoprecipitate substitution therapy.⁷⁵ A few virally inactivated plasma-derived concentrates are available in some countries for the treatment of inherited afibrinogenemia and hypofibrinogenemia such as Clottagen (LFB, France) and Haemocomplettan P (ZLB Behring, Germany). Effective long-term secondary prophylaxis with administration of fibrinogen concentrates every 7–14 days, particularly after CNS bleeds, has been described,^{76,77} although the minimal protective level is not well defined. Fibrinogen concentrates have also been used with some success in acquired disorders including haemodilution from massive post-partum bleeding,⁷⁸ although no firm evidence regarding efficacy and safety, is available.

Factor VII concentrates

There is a poor correlation between FVII levels and the risk of bleeding in FVII-deficient patients.⁷⁹ Replacement therapy has traditionally been achieved using FFP, “four factor” (FVII-enriched) PCCs, or virally inactivated pdFVII concentrates. In the latter category, three such products are available, although none are marketed in the USA. Well-designed clinical studies documenting haemostatic levels of FVII activity in all situations are lacking, although empirically, a target trough activity of at least 10–15 IU/dL (10–15%) is usually recommended.⁸⁰ Recombinant FVIIa is now widely used in these patients.^{81,82} Preliminary reports suggest that FVII concentrates of plasma or recombinant origin can be effective when administered prophylactically.⁸⁴ Develop-

ment of alloantibodies against exogenous FVII is a rare complication.⁸⁴

Factor XI concentrates

FXI deficiency has a variable clinical phenotype with a lack of a clear association between bleeding and FXI coagulant activity.⁸⁵ Bleeding can be excessive after surgery or trauma. While FFP is the only available therapy in the USA, two others, FXI concentrate (Bioproducts Laboratory, Elstree, UK) and Hemoleven (LFB, France), which undergo two viral inactivation steps, are available elsewhere. Retrospective analyses of their use in Europe and Canada have shown them to be safe and effective, although there is a potential for thrombotic complications.^{86,87} In both products, heparin and antithrombin are added in an attempt to minimise this risk.

Factor XIII concentrates

FXIII circulates as a tetrameric protein consisting of two A and two B subunits. FXIII concentrates have been produced from both human plasma and placenta. However, placental FXIII concentrates are no longer available, and the FXIII-A2B2 heterotetramer (Fibrogammin P, ZLB Behring, Germany) is the only plasma-derived concentrate on the market. It has been administered for treatment and prophylaxis of patients with FXIII deficiency.⁹⁰ This concentrate is approved in several markets including Japan and a number of EU countries, but in the USA, only FFP and cryoprecipitate are available for use in FXIII deficiency. The recommended prophylactic doses of 10–35 IU/kg can be administered every 4–6 weeks because of its half-life of 5–11 days. Development of alloantibodies against exogenous FXIII is extremely rare, although very problematic when it does happen.⁹¹

A new recombinant FXIII-A2 (rFXIII-A2) homodimer containing no human or mammalian products in the culture medium has been manufactured in *Saccharomyces cerevisiae*. The rFXIII-A2 homodimers are able to associate in plasma with endogenous FXIII-B

subunits to form the stable heterotetramer FXIII-A2B2. The safety, pharmacokinetics, and immunogenicity of rFXIII-A2 have been studied in healthy volunteers in a phase 1 clinical trial.⁹⁰ This study shows that rFXIII-A2, when combined with endogenous FXIII-B subunits, has a half-life similar to that of native FXIII. No serious adverse events or evidence of antibody formation to yeast or rFXIII have been detected, suggesting that rFXIII represents a safe and effective alternative to pdFXIII in patients with FXIII-A2 deficiency.

FXIII concentrate could also be useful in patients with acquired FXIII deficiency, including after cardiac bypass surgery,⁹¹ stem-cell transplantation and graft-versus-host disease,⁹² and inflammatory bowel disease,⁹³ though the precise benefit of treatment requires further investigation.

Off-label use of rFVIIa

During the past decade, there has been considerable interest in the off-label use of rFVIIa in a variety of acquired medical and surgical haemorrhagic disorders in patients without inherited coagulopathies. The benefit of rFVIIa compared with placebo was shown in retropubic prostatectomy, in which it reduced blood loss and red cell transfusion requirements,⁹⁴ and in spontaneous (non warfarin-related) intracerebral haemorrhage in which it reduced haematoma size and neurological disability.⁹⁵ However, prospective RCTs in trauma,⁹⁶ orthotopic liver transplantation,^{97,98} partial hepatectomy,⁹⁹ and major pelvic surgery¹⁰⁰ all failed to show efficacy. A summary of adverse events reported to the US Food and Drug Administration included 185 thromboembolic arterial and venous events, most of which occurred after the off-label use of rFVIIa.¹⁰¹ This report highlights the need to assess safety as well as efficacy in prospective RCTs of rFVIIa, and to assess the risk-benefit when using rFVIIa for off-label indications.¹⁰²

Clotting factor concentrates in reversing therapeutic anticoagulation

Bleeding from warfarin sodium (4-hydroxycoumarin)-induced over-anticoagulation is a common cause of morbidity and mortality.¹⁰³ The most severe bleeding complication is intracerebral haemorrhage, which has a mortality of at least 50%.^{104,105} When urgent reversal of excessive anticoagulation is needed in a patient who is actively bleeding, the standard of care in many countries continues to be the administration of FFP and vitamin K.^{104–106} This approach is limited by the time delay involved in thawing plasma, the risks (albeit low) for viral transmission and transfusion-associated lung injury (TRALI), and the potential for fluid overload from the large volume of plasma required. These concerns have prompted an examination of the role of PCCs and rFVIIa as alternative approaches to urgent warfarin reversal.^{107–109}

On the basis of studies showing that PCCs reverse warfarin coagulopathy more rapidly and completely than

the standard dose of FFP (10–15 mL/kg), several expert consensus panels have recommended the use of PCCs.^{110–112} A single dose of 30 U/kg or lower seems to be enough to reverse even the most over-anticoagulated patients, with a very low thrombotic risk.^{108–113} These recommendations have not been widely adopted, especially in North America, possibly because of limited licensing for this indication (only for a few products, and only in Europe), or because of lingering perceptions regarding the thrombotic risk associated with these products in haemophilia;^{35,39} however, this is a very different situation with respect to dosing levels and frequency. There is an urgent need for randomised clinical trials correlating clinical outcomes of PCC or rFVIIa vs FFP with correction of INR values in patients with warfarin-induced haemorrhage.

The development of alternative anticoagulants to standard unfractionated heparin and warfarin continues to be an active area of pre-clinical and clinical development.¹¹⁴ These agents are generally targeted inhibitors of specific procoagulants, most commonly factor Xa and/or thrombin. While many of these agents possess desirable pharmacological properties such as a more predictable dose-response relationship and greater convenience, they are universally lacking in a specific antidote for reversal in the event of bleeding. Some evidence exists that rFVIIa is capable of partially restoring intravascular thrombin generation in healthy volunteers treated with the pentasaccharide inhibitors of factor Xa.^{115,116} However, clinical evidence for its efficacy is so far limited to case reports.¹¹⁷

Clotting factor concentrates in inherited and acquired thrombotic disorders

Antithrombin concentrates

Hereditary antithrombin deficiency is associated with a significant risk of venous thromboembolism, and patients with this disorder frequently require long-term anticoagulation. Discontinuation of anticoagulation for childbirth or surgery can carry a substantial thrombotic risk, and replacement with antithrombin concentrate has been proposed in these situations.¹¹⁸

Plasma-derived antithrombin is marketed in many countries, and the various production processes contain at least one viral inactivation step. A new recombinant human antithrombin concentrate (Atryn, GTC Biotherapeutics, Framingham, MA, USA) produced in the milk of transgenic goats was investigated in a pilot study in which five patients with hereditary antithrombin deficiency underwent six surgical procedures. No thrombotic or bleeding complications occurred.¹¹⁹ Despite differences in glycosylation (eg, oligomannose structures) with recombinant human antithrombin that probably account for its altered pharmacokinetics,¹²⁰ this product was recently approved in the EU for the prophylaxis of venous thromboembolism in surgery in patients with congenital AT deficiency.

The use of antithrombin concentrates in acquired deficiency states is disputed. Despite preliminary encouraging results, a pivotal phase III randomised controlled clinical trial of antithrombin concentrate failed to show a beneficial effect on 28-day mortality in adults with severe sepsis.¹²¹ However, a recent post-hoc analysis of this trial showed a significant reduction in mortality in septic patients with overt DIC treated with high-dose antithrombin concentrate in the absence of heparin.¹²² In neonatal respiratory distress syndrome, intracranial haemorrhage, or sepsis, treatment of acquired AT deficiency could improve outcomes, but definitive evidence is lacking.¹²³ In children with acute lymphoblastic leukaemia and acquired antithrombin deficiency associated with the use of L-asparaginase, the group treated with antithrombin concentrate showed a trend to efficacy and safety (incidence of thrombosis 28% [95% CI 10–46%], compared to 37% [95% CI 24–49%] in the non treated arm).¹²⁴

Protein C and activated protein C concentrates

In patients with severe (homozygous) inherited protein C deficiency, including neonates, replacement therapy with human plasma-derived protein C is effective, especially for treating cutaneous thrombosis (purpura fulminans) and preventing thrombosis in high-risk situations.¹²⁵ In patients with moderate (heterozygous) deficiency, a short-course of human protein C prophylaxis may reduce the frequency of thrombosis in high-risk situations.¹²⁶ This drug has also been used for long-term prophylaxis, in inherited homozygous protein C deficiency.¹²⁷

Severe sepsis is associated with rapid depletion of protein C and blunted endogenous protein C activation. Protein C concentrates have been reported to improve outcomes in meningococcal sepsis.¹²⁸ The activated form of human protein C (hAPC) possesses anticoagulant, profibrinolytic, and anti-inflammatory properties. A landmark phase III study in adults with severe sepsis showed that treatment with recombinant hAPC (rhAPC; drotrecogin alfa [activated]) was associated with a 6.1% absolute reduction in 28-day mortality compared with placebo.¹²⁹

However, in a second randomised clinical trial, no efficacy of rhAPC was seen in patients with severe sepsis at a lower risk of death. An increased incidence of serious bleeding complications was seen in rhAPC-treated patients.¹³⁰ Furthermore, a large randomised, placebo-controlled trial with rhAPC in paediatric sepsis was stopped early because of lack of efficacy.¹³¹ Further clinical trials are needed to establish efficacy of rhAPC in the treatment of patients with severe sepsis.

The future

The routine production of coagulation factors by recombinant technology, and the disappointingly slow progress of gene replacement therapy for single gene disorders such as haemophilia, has prompted the development of bioengineered products that have been

mutated to overcome their therapeutic limitations. The proteins of interest are usually modified to enhance their pharmacokinetic properties or reduce immunogenicity. Already, mutant forms of rFVIIa with enhanced activity are under pre-clinical development, and encouraging phase I/II studies confirming the extended protection from bleeding afforded by the weekly infusion of FVIII bound to synthetic polyethylene glycol (PEG)-coated liposomes have been reported.¹³² Various pre-clinical approaches have supported the potential therapeutic value of FVIII modified to enhance its circulating half-life by other means (such as polysialylation), or mutated to enhance its resistance to degradation or clearance.¹³³ B-domain deleted recombinant porcine FVIII molecule is undergoing clinical trials for the treatment of patients with congenital or acquired haemophilia whose inhibitors are only partially cross-reactive to porcine FVIII.¹³⁴

Another unique approach under development for haemophilia B is a synthetic protein comprising FIX fused with the Fc region of IgG to extend the half-life of FIX. A growing trend that is also likely to follow the development of new recombinant clotting factors is their experimental use in acquired deficiency states. However, cost remains a significant limitation of all these technologies. The disparity in the availability of coagulation factor concentrates worldwide is illustrated by the case of haemophilia, where it is estimated that more than 75% of the world's patients with haemophilia receive either inadequate or no treatment whatsoever.¹³⁵ It can only be hoped that the development of transgenic technologies increases the availability and markedly reduces the cost of factor concentrates in the future.

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