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Guidelines on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders

UNITED KINGDOM HAEMOPHILIA CENTRE DOCTORS' ORGANISATION (UKHCDO)

Summary. Evidence based guidelines are presented on the selection and use of therapeutic products to treat haemophilia. These guidelines offer advice based on the best published scientific and medical information. They will be reviewed regularly by UKHCDO Advisory Committee. Included are details of therapeutic products available in the UK to treat patients with haemophilia and other bleeding disorders and the background information on which the recommendations are based are presented.

Keywords: Haemophilia, other hereditary bleeding disorder, therapeutic products.

Introduction

These guidelines are to inform those making choices about coagulation factor concentrates, and other therapeutic products, for treating individuals with heritable coagulation bleeding disorders, principally the haemophilias. We hope, therefore, that the guidance will be of value to physicians, nurses, laboratory scientists as well as patients and those with a responsibility for funding services. This is the fourth edition of the therapeutic guidelines and supersedes the last edition that was prepared in 1996 [1].

Over the past 40 years, haemophilia, if uncomplicated by inhibitor development, has become a condition that is readily treatable with clotting factor concentrates with a resulting normal life expectancy preventing crippling arthritis, muscle atrophy and neuropathies, which previously resulted in many becoming severely disabled by early adult life. By the 1970s, the prospect of transforming lives of patients led to optimism that haemophilia was an eminently and simply treatable condition. However, infections transmitted by the concentrates that were prepared from large pools of plasma from many thousands of individual donors resulted in a series of potentially fatal viral infections that affected a very high proportion of those who had received these otherwise lifesaving therapies. In order to prevent further transmission of blood-borne virus infections, increasingly sophisticated testing of donations and plasma pools and virucidal treatments of the concentrates have been introduced. Current plasma-derived concentrates are manufactured with at least a single viral inactivation process, which is predominantly effective against lipid-enveloped viruses; but it should be remembered that currently available and licensed concentrates may still transmit non-lipid coated, pathogenic viruses. The safety of plasma-derived coagulation factor concentrates, therefore, depends, not just on viral inactivation processes, but also on other aspects like donor selection, plasma pool size as well as the fractionation procedure. The safest possible concentrates should be provided for future clinical use.

During the 6 years since the last guidelines were prepared, some of the changes in treatment have been predictable, like the move towards increasing numbers of patients receiving recombinant concentrates. The first generation of these were manufactured using animal and human proteins in the cell culture medium and albumin, as a protein stabilizer, in the final vial. These are being superseded by the second-generation concentrates in which the albumin in the final vial is replaced by non-protein stabilizers, and third-generation concentrates that lack added animal and human proteins in the cell culture. These enhancements are significant advances in the manufacture and will reduce the risk of recombinant concentrates transmitting infectious agents of animal or human origin.

Some changes have been quite unforeseen, such as the move away from using UK plasma for manufacturing concentrates because of the potential risk that the agent responsible for variant CJD (vCJD;

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Creutzfeldt Jakob disease) might be transmissible [2]. Furthermore, the spread of BSE (Bovine Spongiform Encephalopathy) to other countries, and the concern that blood donors who have lived in high incidence areas may be at risk of transmitting the infectious agent, has led to a reduction in the global availability of plasma. This, along with the extra loss in yield of factor VIII with the introduction of a second viral inactivation step, has led to a marked reduction in availability of plasma-derived concentrates. A balance, therefore, has to be struck between ensuring a secure continuing supply of essential treatment, its safety and cost.

The introduction of virally inactivated plasma, which is now also to be sourced from outside the UK for children born after 31 December 1995, has led to a re-evaluation of the use of fresh frozen plasma (FFP) for treating coagulation disorders.

To monitor the safety of therapy and maintain confidence in products, it has become important, and in many cases a condition of licensing, that there is appropriate surveillance of recipients. The arrangements for this long-term monitoring will become more imperative in future and will be an essential feature of good clinical practice.

Patients with haemophilia are very well informed about their condition, its treatment and the potential hazards of therapy and it is, therefore, appropriate that their views are taken into account when therapeutic decisions are being considered. In addition, the product information leaflet should always be consulted.

Methods

The guidelines were drafted by a Working Party of Haemophilia Centre Directors appointed by the UKHCDO Advisory Committee to represent the Organisations Adverse Events, von Willebrand disease, Inhibitor, Paediatric and Transfusion Transmitted Infection Working Parties. Representatives were also sought from the UK Haemophilia Alliance (representing UK Haemophilia Society, UK RCN Haemophilia Nurses Association, Haemophilia Chartered Physiotherapists Association, Clinical Scientists Group, Institute of Biomedical Science and Haemophilia Social Workers Group), Commissioners of Health Services and the UK Departments of Health. A draft copy of the guideline was widely circulated for consultation. It was also sent for professional review to Royal College of Physicians of London, Royal College of Physicians of Edinburgh, Royal College of Physicians and Surgeons of Glasgow, Royal College of Pathologists, Royal College of Obstetricians and Gynaecologists and Royal College of Paediatrics and Child Health and the responses noted. During the preparation of the guideline, successive drafts were circulated to the UK Departments of Health. The final draft was circulated to coagulation concentrate manufacturers and UK National Transfusion Services.

The information contained in this review was gathered from several sources. These include references known to the Working Party members supported by a search of MEDLINE and the Cochrane databases for randomized trials and systematic reviews comparing treatments of coagulation disorders. Recommendations have been based on reports with the highest levels of evidence (Appendix 3). Members of the Working Party made a declaration of interest to the Chairman UKHCDO.

Therapeutic recommendations

General recommendations

Patient information and consent Good practice dictates that the necessity for treatment is appropriately explained to the patient and/or parent. This should include the advantages and risks of different therapies to allow an informed decision to be made. When consent has been obtained this should be recorded in the case notes.

Vaccination against hepatitis A and B Hepatitis A and B vaccination is highly effective in preventing infection after exposure (Level IIa, Grade B). All patients who currently receive, or may require, blood products should be vaccinated. Carers who are preparing and/or injecting blood products should also be offered vaccination. Hepatitis A vaccine is not licensed for those less than 1 year of age. In patients the vaccines should be given subcutaneously, not intramuscularly, to reduce the risk of haematoma at the injection site and the antibody response assessed (Level IIb, Grade B).

Avoidance of exposure to concentrates, blood products and animal proteins Mild haemophilia A and von Willebrand disease (vWD) should be treated with desmopressin (DDAVP) (and tranexamic acid) in preference to coagulation factor concentrates whenever possible (Level IIb, Grade B).

Choosing a therapeutic product

The key issues in selecting a product are its efficacy and safety, although there are other considerations

like the volume and ease of reconstitution and stability that also need to be considered.

Efficacy Before a licence is granted a product will have to have demonstrated pharmokinetic equivalence to other licensed concentrates, as well as safety and efficacy in the clinical setting. The extent of clinical studies will depend on the novelty of the manufacturing process, but some concentrates prepared by well-established processes may not be required to demonstrate efficacy in a large number of patients.

Safety When selecting a plasma-derived or recombinant concentrate, the two most important safety issues are infectious agents and inhibitor formation (including anaphylaxis). Clinical trials, however, of new products are usually undertaken on relatively few patients and are, therefore, not large enough to assess the incidence of rare complications. A trial, for example, involving 3000 patients cannot rule out a one in 1000 risk with 95% certainty. Safety from rare complications is usually assessed from vigilant follow-up of treated patients in formal post-marketing surveillance studies.

To minimize infectious risk, it is essential to consider the whole manufacturing process for both plasma-derived and recombinant concentrates. If the process contains any human or animal plasma component, the epidemiology of known, and potential, transfusion transmissible infectious agents need to be considered. Thus, the country of origin of the plasma, donor selection, viral screening by antibody, antigen and nucleic acid testing (NAT) techniques, virus removal and inactivation processes (usually demonstrated with model viruses) and the extent of clinical experience with the concentrate should be taken into account. To reduce the chance of infection by exogenous viruses in a recombinant concentrate, consideration should be given to choosing one, where available and licensed, that is manufactured with the least addition of human or animal protein.

The risk and consequence of inhibitor development (and anaphylaxis in haemophilia B) in each individual patient is related to the genetic mutation causing the haemophilia A and B and a product should, therefore, be selected, when appropriate, to minimize this risk.

When choosing a concentrate, reference should be made to the product specification document and further data should be sought, if necessary, from the manufacturer or distributor. Additional information may be available from post-marketing surveillance, which increasingly is a condition of product licence.

Licensed products Licensed products used within their product licence should be preferred to unlicensed products or products used outside their product licence unless there are clear advantages to an alternative treatment (Level IV, Grade C). Unlicensed products should be used, if possible, under formal clinical trial rather than on a named patient basis.

Specific recommendations

Haemophilia A Recombinant FVIII is the treatment of choice. If recombinant FVIII is not available, a plasma-derived concentrate should be used (Level IV, Grade C). When selecting a product from these categories, consideration should be given to the issues discussed above and the general recommendations.

Haemophilia B Recombinant factor IX is the treatment of choice. The only available product is currently not licensed for use in previously untreated patients or in those less than 6 years of age because of lack of clinical data and concerns about the risk of anaphylaxis. For these patients, however, on balance it is also recommended because no exogenous human or animal proteins are used in its manufacture. It must be infused with appropriate care and safeguards because of the risk of anaphylaxis. The alternative to recombinant FIX is a high-purity plasma-derived FIX concentrate. These cause less haemostatic activation than prothrombin complex concentrates [3,4] (Level Ib, Grade A), which should be avoided because of the increased risk of thrombosis.

Coagulation concentrates for treating patients with inhibitors Treatment of these patients includes the treatment of acute bleeds and treatment to induce immune tolerance. The products in addition to FVIII and FIX concentrates recommended for the treatment of patients with inhibitors are given in Table 6. Recommendations on their use have been made in a UKHCDO guideline [5]. Where possible and clinically appropriate, patients with congenital haemophilia should have their bleeds treated with recombinant products.

von Willebrand disease A concentrate containing von Willebrand factor (vWF) is the treatment of choice when DDAVP is not likely to be effective or is contra-indicated (Level IIb, Grade B) (Table 4). Detailed guidance is given in the UKHCDO guideline on management of vWD.

Factor XI deficiency The majority of patients with FXI:C levels <15 U dL^{-1} will suffer excessive

bleeding following trauma or surgery and should be managed with infusions of FXI concentrate [6]. In those with partial deficiency of FXI (15–70 U dL^{-1}), bleeding is more difficult to predict. Where there is a clear history of abnormal bleeding and treatment is required to secure haemostasis, the use of FXI concentrate is justified. The dose of FXI should be sufficient to raise the level of FXI:C to 70 U dL^{-1} and should not exceed 100 U dL^{-1} because of the risk of thrombosis (maximum dose 30 U kg^{-1}) [7,8] (Level IV, Grade C). Where there is no helpful history of bleeding, tranexamic acid may be used alone but in the event of subsequent excessive bleeding must be replaced by FXI concentrate. Patients should be assessed for pre-existing risk of thrombosis and the concentrate should be used with great caution in those with a history of cardiovascular disease (Level IV, Grade C). Virally inactivated plasma is recommended when FXI concentrate is contra-indicated.

Factor VII deficiency Recombinant VIIa is the treatment of choice although it is not licensed for this indication (Level IV, Grade C). If a plasma-derived concentrate is used, then a specific plasma-derived FVII concentrate should be favoured over a FVII containing prothrombin complex concentrate because of the increased risk of thrombosis with the latter.

Factor II or X deficiency No specific concentrates are available and prothrombin complex concentrates are the treatment of choice (Level IV, Grade C).

Factor V deficiency Factor V containing concentrates are not available and plasma is the only available treatment. Virally inactivated plasma is recommended.

Factor XIII deficiency Factor XIII concentrate is the treatment of choice (Level IV, Grade C).

Fibrinogen deficiency Fibrinogen concentrate is the treatment of choice (Level IV, Grade C).

Background information on which the recommendations are based

Recombinant concentrates (Table 1)

In order to manufacture recombinant coagulation factors, the gene (or a modified gene) needs to be inserted into a cell line. The cells are cultured and the secreted factor is purified from the culture medium. The factor must be stable throughout the production process and in the final formulation for lyophilization. Concern has been expressed over the use of human and animal products in the culture media, and over the use of human albumin as a stabilizer. If mouse monoclonal antibodies are used in the purification process, trace amounts may appear in the final product. There is the possibility of viral infection of the cell lines used to produce the coagulation factor and any monoclonal antibodies used. The orbivirus bluetongue virus, which infects primarily sheep, occasionally goats and deer and, very rarely, cattle, has infected cell culture lines including those from Chinese hamster ovary (CHO) [9]. CHO cells have also been contaminated by minute virus of mice, a parvovirus [10]. These arise from contamination but CHO cells can also produce endogenous retroviral particles [10], which suggests that even if all animal and human proteins can be removed from the production process a viral inactivation/removal step will enhance safety. A further consideration is that if a new mutation occurred in the coagulation factor gene during cell culture an aberrant defective coagulation factor could be produced and if more immunogenic then the wild type coagulation factor may result in a higher incidence of inhibitors.

First-generation recombinant coagulation factor concentrates First-generation products have human albumin added to the final formulation as a stabilizer. Two first-generation preparations of recombinant FVIII were licensed in the early 1990s, Kogenate

Table 1. Summary of recombinant products available.

	Cell line	Gene	Protein in culture medium	Murine mAbs	Human albumin as stabilizer	Viral inactivation removal	Generation
Recombinate	CHO	VIII vWF	Bovine albumin, insulin, aprotinin	Yes	Yes	No	1
Helixate Kogenate Bayer	BHK	VIII	Human albumin	Yes	No	SD	2
Refacto	CHO	B-domain deleted VIII	Human albumin	Yes	No	SD	2
NovoSeven	BHK	VII	Bovine serum	Yes	No	SD	2
Benefix	CHO	IX	No	No	No	NF	3

BHK, baby hamster kidney; CHO, Chinese hamster ovary. SD, solvent detergent; NF, nanofiltration; mAbs, monoclonal antibodies.

(Bayer) [also labelled as Helixate (Aventis-Behring)], which is no longer manufactured, and Recombinate (Baxter).

Second-generation recombinant coagulation factor concentrates Two newer recombinant FVIII preparations stabilized without the addition of human albumin are referred to as second-generation products, Kogenate Bayer (Bayer) [also labelled as Helixate Nextgen (Aventis Behring)] and ReFacto (Wyeth); both have human albumin in the cell culture medium. Recombinant VIIa (Novoseven, NovoNordisk) does not contain any stabilizing protein, although bovine serum is used in the cell culture medium.

Third-generation recombinant coagulation factor concentrates In third-generation products, animal products have been removed from the culture media. Two recombinant FVIII products, R-PFM (Baxter) and ReFacto AF (Wyeth), manufactured and formulated without human or animal protein are under clinical trial; the latter does not use monoclonal antibodies in its preparation. The only recombinant FIX, Benefix (Baxter), has no human or animal protein used in its preparation or formulation.

Plasma-derived concentrates

Plasma-derived concentrates have allowed the successful treatment of bleeding episodes since their introduction. The history of their use has, however, been complicated by transfusion-transmitted infection, which has resulted in an on-going need to review and improve the processes involved in their production.

The reduction of risk of transfusion-transmitted infection is a multi-step process. Selection of donors and testing of donations to minimize contamination of a plasma pool is combined with virus removal during fractionation, and specific virus separation and inactivation processes to produce as safe a product as possible. In order for these combined processes to succeed, knowledge of the epidemiology of known and potential new blood-borne infections in the population combined with on-going surveillance of donors is essential. Attention to documentation and Good Manufacturing Process is an integral part of this process.

Plasma source The description of vCJD and its association with BSE has resulted in a change in policy regarding the use of plasma for fractionation in the UK. As a result, all plasma-derived concentrates produced in the UK since 1998 have been manufactured from the plasma of European and USA donors. Variant CJD has been described in countries other than the UK and the identification of BSE-infected cattle in other countries demands that plasma sources are continually reviewed.

In view of the epidemiology of BSE and its association with vCJD, the FDA has introduced criteria for a stepwise deferral of donors with a history of prolonged residence in Europe between 1980 and 1996 or receipt of a blood transfusion in the UK from 1980 to the present.

Donor screening/testing All plasma donations used in products available in the UK are individually tested for anti-HIV1 and 2, HBsAg and anti-HCV using assays approved by the regulatory authority of the country of origin and fractionation. Anti-HTLV1 screening of donations is also being introduced into the UK. In an attempt to improve the detection of infected donations, routine testing of plasma pools for HBsAg, anti-HIV and anti-HCV has been introduced (EEC Ad Hoc Working Party on Biotechnology/Pharmacy 1994). Further reduction in the viral load of plasma pools can be achieved by NAT and exclusion of positive pools. Since July 1999, an EEC directive has demanded that manufacturing plasma pools be tested for HCV RNA. Plasma pools for each batch are also checked by NIBSC or other Medicines Control Laboratories.

Fractionation The combination of different fractionation processes used in the preparation of coagulation factor concentrates account, in part, for the purity of the final product both in terms of the concentration of the target protein and reduction in its infectivity. Separation techniques may also reduce the 'load' of the other possible infectious agents such as prions. Data from experiments using rodent models of transmissible spongiform encephalopathies (TSE) need to be interpreted with care, however, as there can be no assurance that the animal prion forms used in these experiments behave like the human prion form in large-scale manufacturing [11]. The FVIII and FIX products available in the UK are produced using combinations of precipitation techniques, ion-exchange chromatography and affinity chromatography. Precipitation techniques exploit differences in protein solubility, while ion-exchange chromatography depends on differences in net charge between different proteins. Affinity chromatography specifically captures target proteins by using an immobilized bio-specific ligand such as a monoclonal antibody.

Virus inactivation/removal The virus inactivation processes used specifically to destroy viruses in concentrates are based on heat, solvent detergent (SD) treatment or filtration. Heat treatment denatures viral proteins and nucleic acids preventing replication and can be performed in the dry or liquid (pasteurization) state or under pressure. SD treatments are effective in disrupting lipid-enveloped viruses such as HIV, HBV and HCV, rendering them non-infectious. The major limitation of the SD treatment is lack of activity against non-enveloped viruses such as hepatitis A and parvovirus B19 [12,13]. Ultrafiltration (nanofiltration) is often used in conjunction with a virus inactivation process as this removes viruses, including non-enveloped ones, on the basis of size alone. Only small coagulation molecules such as FIX and FXI can be purified in this way because of the small size of the pores.

All virus inactivation and removal procedures have their limitations. It is recommended that two distinct and effective steps that are complementary be incorporated into the plasma product manufacturing process [14]. European guidelines recommend that at least one step effectively inactivates or removes non-enveloped viruses. A Committee for Propreietary Medicinal Products (CPMP) recommendation of 2001 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 physico-chemical 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 non-enveloped viruses. Where a process step is shown to be reliably effective in inactivating/removing a wide range of viruses including enveloped and non-enveloped viruses of diverse physico-chemical characteristics and the process contains additional stages reliably contributing to the inactivation/removal of viruses, a second effective step would not be required.'

Coagulation factor concentrates available in the UK

The concentrates available have been tabulated in alphabetical order for treatment of haemophilia A (Table 2), haemophilia B (Table 3), vWD (Table 4), rarer coagulation disorders (Table 5) and inhibitor patients (Table 6). Details of different preparations of FFP are given in Table 7. Further details of each of the concentrates and other therapeutic products, eg desmopressin and tranexamic acid, have been set out in Appendices 2 and 3.

Safety data on which recommendations are based

Transfusion transmitted infection

Since efficacious products to prevent and control bleeding have been available, the reason to change manufacturing processes and ultimately to move to the use of recombinant products has been transfusion transmitted infection. Large numbers of patients treated with plasma-derived concentrates previously contracted HIV, hepatitis B and hepatitis C. The introduction of specific virus inactivation steps has markedly improved the safety of plasma-derived concentrates. Early inactivation procedures were sub-optimal but since the introduction of terminal dry heat treatment at 80 °C for 72 h, pasteurization at 60 °C for 10 h and SD treatment using a non-volatile organic solvent, tri(*n*-butyl) phosphate in conjunction with a detergent such as Tween-80 or Triton X-100, no case of HIV transmission has been reported. The safety and efficacy of these methods in preventing transmission of hepatitis B and C has also been demonstrated in prospective studies. Although isolated incidents of likely transmission of hepatitis B or C have been reported after the use of pasteurized concentrates, there is no reported transmission of hepatitis B or C by products virus inactivated by SD or terminal dry heat treatment [15].

Infection with protein-coated viruses, which are partially or wholly resistant to these processes, remains a problem. Hepatitis A was transmitted by a SD-treated concentrate in the early 1990s [13] and parvovirus B19 appears to be relatively resistant to all currently available inactivation techniques [12,16]. Porcine parvovirus has been detected in porcine FVIII, but although there is no evidence that it has been transmitted to humans, this concern has led to the withdrawal of the product.

Concerns remain about the risk of transmission of new infectious agents in human plasma, cell cultures, monoclonal antibodies and other animal products despite a lack of any scientific evidence for any transmission.

CJD and vCJD

Despite the use of concentrates for over 40 years, no case of clinical CJD has been reported in a patient with haemophilia. The risk of transmission of clas-

Table 2. Factor VIII concentrates available in the UK.

Product (manufacturer)	Product licence	Manufacturing process	Viral inactivation	Source plasma	Storage
Recombinant					
Kogenate (Bayer)	Yes	Cultured BHK cells	SD		2–8 °C. Do not freeze
Helixate NexGen (Aventis Behring)	Yes	Cultured BHK cells	SD		2–8 °C. Do not freeze. May be kept at up to 25 °C for up to 2 months, but product must then be discarded if unused
Recombinate (Baxter)	Yes	Cultured CHO cells	None		May be stored 2–8 °C for up to 24 months. Do not freeze. May be kept at up to 15–25 °C for up to 6 months in this period
Refacto (Wyeth)	Yes	Cultured CHO cells	SD		Store at 2–8 °C. May be kept for one single period of up to 3 months at room temperature (not above 25 °C)
Plasma derived					
8Y (BPL)	Yes	Addition of glycine/NaCl to cryoprecipitate	Dry heat (80 °C/72 h)	USA	2–8 °C. Do not freeze. Short periods of storage up to 2–3 months at room temperature will not damage the product
Alphanate (Alpha)	Yes	Heparin ligand chromatography	SD and dry heat (80 °C/72 h)	USA	2–8 °C. Do not freeze. May be stored at room temperature (not above 30 °C) for up to 6 months
Beriate P (Aventis Behring)	Yes	Ion-exchange chromatography	Pasteurized (60 °C/10 h)	Austria, Germany and USA	2–8 °C. Do not freeze
Fanhdi (Grifols)	Yes	Heparin ligand chromatography	SD and dry heat (80 °C/72 h)	USA	2–8 °C. Do not freeze
Hemofil-M (Baxter)	Yes	Monoclonal antibody purification	SD	USA	Up to 24 months at 2–8 °C. May be stored at not more than 30 °C for 6 months within this period
Liberate (SNBTS)	Yes	Ion-exchange chromatography	SD	USA and Germany	2–8 °C. May be stored up to 1 month up to 25 °C. Do not freeze
Monoclate-P (Aventis Behring)	Yes	Monoclonal antibody purification	Pasteurized (60 °C/10 h)	USA	2–8 °C. Do not freeze
Replenate (BPL)	Yes	Monoclonal antibody purification	SD	USA	2–8 °C. Periods of storage, up to 24 months, at ambient temperature (25 °C) are not detrimental

sical CJD by transfusion has been extensively investigated and the risk is currently only theoretical [17].

Variant CJD is a novel form of TSE first recognized in 1996 [18] and is associated with the same transmissible agent responsible for BSE. Variant CJD is thought to have occurred through the consumption of contaminated bovine food products. There is evidence that the TSE agent that causes vCJD is more invasive to the lymphoreticular tissue than classical CJD [19] and therefore there is a new, but hypothetical, risk of infection through treatment with blood and blood products including coagulation factors. Experimental evidence exists of transmission by transfusion of blood from pre-symptomatic and symptomatic infected sheep to non-infected sheep [20,21].

Without a reliable diagnostic test for the selection of donors and testing of plasma-derived blood products, a donor deferral policy remains the main preventative measure [17].

Inhibitors

Retrospective studies have shown a prevalence of FVIII inhibitors of 6–20%. However, a higher incidence has been found in prospective studies of

Haemophilia (2003), 9, 1–23

Table 3. Factor IX concentrates available in the UK.

Product (manufacture)	Product licence	Manufacturing process	Viral inactivation	Plasma source	Storage
Benefix (Wyeth)	Yes (except PUPs and subjects under age of 6)	Recombinant: cultured CHO cells	Nanofiltration	Not applicable	2–8 °C. Do not freeze. May be removed from such storage for maximum of 1 month up to 25 °C but should then be discarded if not used
Alphanine (Alpha)	Yes	Ion-exchange and carbohydrate ligand chromatography	SD and nanofiltration	USA	2–8 °C. Do not freeze. May be stored at room temperature (not above 30 °C) for up to 3 months
HIPFIX (SNBT)	Yes	Ion-exchange and heparin ligand chromatography	SD and dry heat (80 °C/72 h)	USA and Germany	2–8 °C. Do not freeze
Mononine (Aventis Behring)	Yes	Immunoaffinity chromatography	Ultrafiltration	USA	2–8 °C. Do not freeze. May be stored at room temperature up to 25 °C for one single period of up to 1 month
Replenine-VF (BPL)	Yes	Metal chelate chromatography	SD and nanofiltration	USA	2–8 °C. Do not freeze. Storage up to 3 months at room temperature (25 °C), in the dark, will not damage product

PUPs, previously untreated patients; SD, solvent detergent.

Table 4. Concentrates containing von Willebrand factor.

Product (manufacturer)	Product licence	Manufacturing process	Viral inactivation	Plasma source	Storage
8Y (BPL)	Yes	Addition of glycine/NaCl to cryoprecipitate	Dry heat (80 °C/72 h)	USA	2–8 °C. Do not freeze. Short periods of storage of up to 2–3 months at room temperature will not damage the product
Alphanate (Alpha)	Yes	Heparin ligand chromatography	SD and dry heat (80 °C/72 h)	USA	2–8 °C. Do not freeze. May be stored at room temperature (not above 30 °C) for up to 6 months
Fanhdi (Grifols)	No	Heparin ligand chromatography	SD and dry heat (80 °C/72 h)	USA	2–8 °C. Do not freeze
Haemate P (Aventis Behring)	Yes	Glycine/NaCl treatment of cryoprecipitate	Pasteurized (60 °C/10 h)	Germany, Austria and USA	2–8 °C. Do not freeze
vWF concentrate (LFB)	No	Ion- and affinity-exchange chromatography	SD	France	2–8 °C. Do not freeze

SD, solvent detergent.

Table 5. Concentrates for other congenital bleeding disorders.

Product (manufacturer)	Product licence	Manufacturing process	Viral inactivation	Plasma source	Storage
Fibrinogen (SNBTS)	No	Multiple precipitation and ion-exchange chromatography	SD and dry heat (80 °C/72 h)	USA and Germany	Store at less than 25 °C
Haemocomplettan HS (fibrinogen) (Aventis Behring)	No	Multiple precipitation	Pasteurized (60 °C/20 h)	USA, Germany and Austria	2–8 °C. Do not freeze
Recombinant VIIa	No	See Table 1	SD	Bovine serum	
Factor VII (BPL)	No	Ion-exchange chromatography	Dry heat (80 °C/72 h)	USA	2–8 °C. Do not freeze. May be stored for up to 1 week at 25 °C
Factor VII (Baxter)	No	Ion-exchange chromatography	Vapour heat: (60 °C/10 h, 190 mbar + 80 °C/1 h, 375 mbar)	USA, Austria, Germany, Sweden and Czech Republic	2–8 °C. Do not freeze
Factor VII (LFB)	No	DEAE absorption + anion-exchange chromatography	SD	France	2–8 °C. Do not freeze
Factor XI (BPL)	No	Affinity heparin sepharose chromatography	Dry heat (80 °C/72 h)	USA	2–8 °C. Do not freeze. May be stored for up to 1 week at 25 °C
Hemoleven (XI) (LFB)	No	Dialysis + cation-exchange chromatography	SD + nanofiltration	France	2–8 °C. Do not freeze
Fibrogammin HS (FXIII) (Aventis Behring)	No	Multiple precipitation	Pasteurized (60 °C/10 h)	USA, Germany and Austria	2–8 °C. Do not freeze
Beriplex PN (Aventis Behring) (FII, FVII, FIX, FX)	No	DEAE–Sephadex	Pasteurized (60 °C/10 h) + nanofiltration	USA, Germany and Austria	2–8 °C. Do not freeze
Profilnine SD (Alpha) (FII, FIX, FX)	No	DEAE–cellulose	SD	USA	2–8 °C. Do not freeze
HT DEFIX (SNBTS) (FII, FIX, FX)	Yes	Ion-exchange chromatography	Dry heat (80 °C/72 h)	USA and Germany	2–8 °C. Do not freeze
Prothromplex-T (Baxter) (FII, FVII, FIX, FX)	No	Ion-exchange adsorption	Vapour heat (60 °C/10 h, 190 mbar + 80 °C/1 h, 375 mbar)	USA, Austria, Germany, Sweden and Czech Republic	2–8 °C. Do not freeze

SD, solvent detergent.

Table 6. Products, in addition to FVIII and FIX concentrates, for the treatment of patients with haemophilia and inhibitory antibodies.

Product (manufacturer)	Product licence	Manufacturing process	Viral inactivation	Plasma source	Storage
Hyate:C (Ipsen)	Yes*	Polyelectrolyte fractionation of porcine plasma	None	UK pigs	Frozen at −15 to −20 °C
FEIBA (Baxter)	Yes	Batch-controlled surface activation of prothrombin complex concentrates	Vapour heat (60 °C/10 h, 190 mbar + 80 °C/1 h, 375 mbar)	USA, Austria, Germany, Sweden and Czech Republic	2–8 °C. Do not freeze. May be stored for 6 months at room temperature (max. 25 °C)
NovoSeven (NovoNordisk)	Yes	Recombinant: made with BHK cells	SD treatment	Not applicable	2–8 °C. Do not freeze

*Currently in the UK, Hyate:C is only available on a 'named patient basis' for life or limb threatening bleed.

Table 7. Fresh frozen plasma.

Product (manufacturer)	Product licence	Viral inactivation	Plasma source	Storage
Octaplas (Octapharma)	Yes	SD	Pooled plasma: each batch contains donations from approx. 1500 donors. Plasma obtained from Austria, Germany and USA	Store at less than −30 °C and protect from light. Shelf life: 2 years
Fresh frozen plasma (NBA and SNBTS)	NA	Methylene blue/ ultraviolet light	Single donors in UK: available for paediatric patients only at present	Store at less than −35 °C. Shelf life: 1 year

SD, solvent detergent.

25–28% although many inhibitors are transient [22,23]. The rate of inhibitor formation is similar for both plasma-derived and recombinant products. FVIII anitgenicity is dependent upon the manufacturing process and some methods have resulted in increased inhibitor production in the recipients [24,25]. Surveillance is, therefore, advised following change of product.

In haemophilia B, studies demonstrate an inhibitor prevalence of 1–3% arising after a median of 11 (range 2–180) exposure days. A unique feature of the inhibitors seen in severe haemophilia B is that inhibitor development is associated with anaphylaxis or severe allergic manifestations on exposure to any FIX containing product in 50% of patients [26]). It is, therefore, advised that the first exposures to products are in the hospital setting with available treatment for anaphylaxis.

Purity

Purity is defined as IU FVIII mg^{-1} protein in plasma-derived concentrates and ranges from 5.0 (intermediate purity) to 2000 (high purity). There are few data to suggest the benefit of high-purity plasma-derived FVIII concentrates over intermediate purity concentrates. There is anecdotal evidence for reduction in allergic reactions associated with the use of high-purity materials. Several studies suggest benefit in HIV disease progression with high-purity products produced using immunoaffinity chromatography [27,28] but these findings were not confirmed in a further study [29] and changes in CD4 counts were not associated with reduction in progression to AIDS or death [30]. Furthermore, any such effects of high-purity concentrates are insignificant compared to the immune reconstitution produced by highly active antiretroviral therapy (HAART).

Thrombosis

All currently used bypassing agents (PCCs, APCCs and rFVIIa) for the treatment of patients with high-titre inhibitory antibodies carry the risk of thrombotic complications, including thromboembolism, disseminated intravascular coagulation (DIC) and myocardial infarction [31]. These complications are rare [32] and are considered to be caused by an increase in the concentration of native, or activated, coagulation factors in the recipient. Thrombotic problems occur most commonly in patients with underlying atherosclerotic disease and those immobile for long periods. Patients with pre-existing liver disease and premature infants are particularly susceptible to DIC when treated with PCCs. Purified FIX concentrates have been shown to have lesser effects on sensitive markers of coagulation than PCCs [33].

Factor XI concentrates Use of FXI concentrates, particularly in high doses, has been associated with thrombosis [6,7]. Elderly patients and those with a previous history of thrombosis or ischaemic heart disease are particularly at risk [8].

von Willebrand-containing factor concentrate Use of a vWF-containing concentrate for the treatment of vWD may result in a high plasma FVIII level, which is known to be a risk factor for thromboembolism [34].

Venous thromboembolism has been reported following the use of vWF-containing concentrates [35,36].

Storage, reconstitution and infusion of concentrates

Storage

Clotting factor concentrates are administered both in hospital and at home and must be transported, handled and stored under conditions to minimize loss of activity. Recommendations for each product should be followed (Tables 2–7).

Reconstitution

All clotting factor concentrates should be reconstituted using the diluent provided and not further diluted. The vials should be gently swirled, avoiding shaking, until the material has dissolved. The filter needle supplied should be used for drawing up the solution into a syringe. Most products carry a recommendation from the manufacturer that they should be used immediately following reconstitution, or within 3 h, to avoid microbiological contamination.

Continuous infusion of clotting factor concentrate

Whilst all clotting factor concentrates can be administered as bolus injections, there has been an increasing interest in delivering treatment by continuous infusion. There are, however, no products currently licensed for this route of administration. The rationale for adjusted continuous infusion is that a steady state, avoiding the peaks and troughs in clotting factor levels seen with bolus injection regimes, may reduce the amount of concentrate used and be more convenient.

Important safety considerations for the use of a product for continuous infusion are stability after reconstitution and risk of bacterial contamination. Most concentrates have acceptable stability for 24 h at room temperature, in some instances for several weeks [37,38]. Stability data for individual products should be sought from the relevant manufacturer. Further dilution than recommended by the manufacturer may result in loss of activity because of dilution of the stabilizer or to adsorption to the plastic walls of container and tubing [39].

Constant infusion of clotting factor causes local irritation to peripheral veins with ensuing thrombophlebitis [40]. Preventative approaches are either the addition of unfractionated heparin to the concentrate or running a parallel normal saline infusion. Neither standard nor low molecular weight heparin should be added to rVIIa [40]. Although some case reports suggest that continuous infusion may be associated with inhibitor formation, there is insufficient evidence to recommend against this form of delivery [41–43].

Labelling of concentrates and assay post-infusion

General principles of standardization

Assays of coagulation factors have been standardized by the establishment of International Standards by the World Health Organization (WHO). These standards define the International Unit (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 patients' plasma samples and hence all such measurements can be made in IU. When the first International Standards for each coagulation factor were established, they were calibrated against fresh normal plasma from a large number of donors, hence 1 IU is approximately equivalent to the amount of each factor in '1 mL of average normal plasma'.

An important principle in biological standardization is that of 'like vs. like'. For many biological substances, not just coagulation factors, reproducibility between laboratories, and between assay methods, is greatest when test and standard are of similar composition. Thus, it has been found in several collaborative studies that plasma standards are unsuitable for assay of coagulation factor concentrates and vice versa [44,45].

Hence, for all the main coagulation factors there are two WHO standards, one for the assay of therapeutic concentrates and another for assay of plasma samples.

Labelling of concentrates

Factor VIII The current (sixth) WHO standard was prepared from a full-length recombinant product, containing albumin, but is used to calibrate both plasma-derived and recombinant products. Other standards are the US Mega and EP standards, which are working standards calibrated against the WHO standard in multicentre 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 US, 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 only used by a few manufacturers. Most US manufacturers use the one-stage method, and the two full-length recombinant products are assayed with this method. The B-domain deleted product, ReFacto, is assayed by the chromogenic method, and most European manufacturers of plasma-derived concentrates use the chromogenic method, which is the recommended method of the EP and of the ISTH [46].

For the full-length recombinant products, differences between potencies by the one-stage and chromogenic methods are less than 10% [47]. However, much larger differences are found for the B-domain deleted product, ReFacto. The manufacturers have reported that the one-stage method gives only 50% of the potency of the chromogenic method, although other laboratories have found smaller discrepancies, with one-stage values of 65–75% of those by the chromogenic method [48]. This has important consequences for the assay of post-infusion samples after treatment with this product (see subsequent section).

For plasma-derived products, the major discrepancies are found with the 'Method M' products, produced by the Baxter Hemofil M process; one-stage assays give potencies around 25–30% higher than those by the chromogenic method [49]. The two products available in the UK are assayed by the two-stage (Replenate) and chromogenic (Hemofil M) methods.

Factor IX The WHO standard is a high-purity single FIX plasma derived product. The US and EP standard share the same large batch of standard as the WHO and thus all products are assayed directly or indirectly against the same standard.

All manufacturers use variations of the one-stage method, and there are no problems of comparability of different products and assay methods for FIX activity.

von Willebrand factor Following licensure of a number of products for treatment of vWD in the USA and Europe during the last few years, all manufacturers of such products have to declare the vWF content of their product on the label. A WHO plasma standard for vWF has existed since 1981, but was found to be unsuitable for assay of therapeutic concentrates. Accordingly, a new WHO standard for vWF concentrate has recently been established, and this is now used by manufacturers to calibrate their products. The WHO standard is calibrated for vWF:Ag and vWF:RCo. The collagen binding method was also investigated but was found to be unsuitable for assay of concentrates because of wide variability according to the different types of collagen used [50].

BPL's 8Y product is labelled for vWF antigen content, but most other products, including the ones available in the UK, are labelled for vWF:RCo activity.

Prothrombin complex There is a WHO standard for FII and FX concentrates and this is used by manufacturers to calibrate these factors in both three, and four, factor concentrates, though not all manufacturers display the values of the individual factors on the label. There is a separate WHO standard for FVII concentrate and this is used for assay of FVII both in four-factor concentrates and in single FVII concentrates.

Most manufacturers use various one-stage methods and there are no major problems with different methodologies, although the assay of FVII can be quite variable depending on the thromboplastin reagent used.

Factor VIIa There is a WHO standard for FVIIa and this is used by the manufacturers to calibrate the specific activity of successive batches of 'Novoseven'. Despite some inter-batch variation in specific activity, labelling and dosage of the product is in mass units, not activity units.

Fibrinogen This is primarily a component of fibrin sealant kits, but is also available as separate concentrates. A WHO standard for fibrinogen concentrate is available and is calibrated for total and clottable protein, the two parameters for which this product is labelled.

Thrombin This is the second component of fibrin sealant kits. Two widely used standards are available: the WHO standard calibrated in IU and the US standard calibrated in NIH units. The two units differ by 10–15%, depending on which assay method is used. The two manufacturers whose products are licensed in the UK both use the WHO standard to calibrate their thrombin. The current WHO and US standards will shortly be replaced by a single batch of thrombin calibrated with a common unitage.

Factors XI and XIII There are no WHO standards for these products, although concentrate standards for both factors are under development. In the meantime, manufacturers use large donor plasma pools to assay their products.

Post-infusion factor level in plasma samples

Assays of plasma samples following infusions of concentrates are carried out for pharmacokinetic studies or to check the haemostatic level of the patient, especially before and after surgery. As these samples consist of plasma, they are normally assayed against plasma standards, which may be local pools, commercial standards, or, in the UK, British Standards, which are available from NIBSC. All these plasma standards are calibrated against the appropriate WHO plasma standards, and so the results can be reported in IU – a convenient way of reporting is IU dL^{-1}, which is numerically equivalent to percentage of normal.

Post-infusion plasmas can, however, also be considered as concentrates 'diluted' in the patient's deficient plasma, and as such a concentrate standard, diluted in deficient plasma, may be more appropriate. This approach, based on the 'like vs. like' principle, has recently been investigated for FVIII, and as a result of this it is now recommended that a concentrate standard is used in assays of the B-domain deleted recombinant FVIII (ReFacto).

Factor VIII Collaborative studies have shown wide variability between laboratories when assaying post-infusion samples, both within each method, and sometimes between methods. For full-length recombinant products, potencies by the chromogenic method have been found to be 20–25% higher than by the one-stage method [51]; when expressed as *in vivo* recoveries, values are usually around 100% by the one-stage method and higher by chromogenic. For Refacto the difference may be much larger, with one-stage assays tending to underestimate the recovery, and there is also wide variability among different versions of both one-stage and chromogenic assays [47].

For full-length recombinant products, the use of concentrate standards has been shown to abolish the difference between the methods, and this approach may be preferable for pharmacokinetic studies. For clinical purposes, the one-stage method with a plasma standard is sufficiently reliable. For ReFacto post-infusion samples, however, assays with plasma standards are unreliable, and a concentrate standard, supplied by the manufacturers, should be used [52]. Using this standard, pre-diluted in deficient plasma, any one-stage or chromogenic method can be used.

Discrepancies between assay methods have also been reported for plasma-derived concentrates, but these are generally of lesser magnitude and less consistent than for the recombinant products; for clinical purposes plasma standards give sufficiently reliable results [44,45].

Factor IX WHO plasma standards are available for use in FIX assays. Data from pharmacokinetic studies has demonstrated reduced recovery using recombinant FIX compared to plasma-derived FIX [53]. This difference appears most marked in young children and emphasizes the need for monitoring.

von Willebrand factor Assays of vWF:Ag and vWF:RCo can be performed using the appropriate plasma standard [50]. Collagen binding assays are not suitable for the same reasons as given for the concentrates.

Factor VIIa Assays of FVII and FVIIa in patients' plasma may be undertaken following infusion of recombinant FVIIa [54]. A specific standard exists for FVII assays. If FVIIa assays are undertaken, a standard of FVIIa (available from the manufacturer or from NIBSC) should be used as plasma standards give unreliable results because of differences in the various thromboplastin reagents used.

Other coagulation factors There are no major problems with the assay of post-infusion plasmas for the other coagulation factors. WHO plasma standards are also available for FII, FX, and fibrinogen, but not for FXI and FXIII; for the latter, large pools of normal plasma should be used as standards.

Licensing and surveillance

Information about the regulations governing the licensing of medicines in the UK can be obtained from the Medicines Control Agency (MCA)

Information Centre (tel.: +44 207 273 0000) or accessed from the MCA website at www.mca.gsi.gov.uk. The MCA assists Ministers of Health (The Licensing Authority) in discharge of responsibilities under the Medicine Act 1968 and relevant European Union (EU) Directives.

Processing of applications for product licensing can be either national, as part of mutual recognition, or centralized under the European Medicines Evaluation Agency (EMEA, www.emea.eu.int) or the Committee for Proprietary Medicinal Products (CPMP).

Applications for licensing products for treating patients with bleeding disorders are reviewed by the Biologicals Sub-Committee reporting its recommendations to the Committee on Safety of Medicines (CSM).

The following summarizes the legal arrangements under which drugs, including coagulation factor concentrates, may currently be prescribed.

Product licence (PL)

The Licensing Authority will grant this for a product if it is satisfied with the safety, quality and efficacy data. The approved indications, doses and approved routes of administration are set out in the Summary of Product Characteristics. The manufacturer bears liability for the production of the drug. A condition of the UK licences for all plasma-derived coagulation factor concentrates is that each batch must be submitted to NIBSC or an equivalent European Medicines Control Laboratory for independent testing before being allowed on to the market. This requirement does not apply to recombinant concentrates as batch release of recombinant products is not allowed under EU legislation.

Clinical trial certificate (CTC)

As the Medicines Act 1968 requires considerable documentation from the manufacturer before issuing a CTC, this procedure has now been replaced by the CTX under the Medicines Exemption from Licences (Clinical Trials Orders, SI 1995 2808 and SI 1995 2809).

Clinical trial exemption (CTX)

The issue of a CTX indicates that the Licensing Authority has not objected to the trial that it does without reference to the Committee on Safety of Medicines. The trial is initiated by the manufacturer who bears liability for the product and usually provides indemnity for patients under the 'good faith' interpretation of the ABPI Guidelines.

Doctors and dentists scheme (DDX)

These provisions apply to the trial of an unlicensed drug by a doctor or dentist (not the drug company). The doctor or dentist bears liability for both the manufacture of the drug and the trial. Local ethical approval is required.

Named patient basis

When an unlicensed drug is prescribed by a doctor without a CTC or CTX, usually to treat an individual patient, this is on a 'named patient basis'. The doctor will bear liability for the prescription and clinical use of the drug. The distributor may be covered by the Consumer Protection Act, 1987. It is recommended, however, that appropriate indemnity is obtained from the manufacturer or distributor, or its agent, prior to clinical use. Hospital Trusts are now drawing up rules for the use of unlicensed drugs, or licensed drugs for unlicensed purposes. Clinicians should, therefore, seek permission from the appropriate authority in the Trust to ensure their protection through Crown Indemnity. Before using a drug on a 'named patient basis', the practitioner must satisfy himself/herself that its use is reasonable and in the interest of the patient. In the event of an adverse reaction, he/she may be called upon to justify his/her actions. The doctor should explain to the patient that the drug is unlicensed and that its use is experimental; he/she should be advised that the extent and severity of contra-indications and side-effects may still not be fully appreciated. This basis for prescribing may be appropriate when there is no licensed suitable alternative.

When a drug with a PL is prescribed for a non-licensed indication, it is prudent to assume that it will be issued on a 'named patient basis' with the responsibility that this implies.

The MCA post-licensing division is responsible for pharmacovigilance, variations to licenses and renewal and reclassification of licenses. The monitoring of safety and quality of medicines includes the Yellow Card Scheme (for further information email: info@mca.gsi.gov.uk) for notifying suspected adverse drug reactions. This system should be used in addition to the UKHCDO's own notification with the orange cards.

Clinical audit

The regular assessment of therapeutic practice by audit is an essential component of good haemophilia practice. Adherence to these guidelines may be audited in many ways, for example:

(1) Are patients receiving the appropriate recommended product?
(2) Are there appropriate pharmacosurveillance arrangements in place, eg screening to detect viral infection and inhibitor formation?
(3) Are there clear local arrangements to ensure that patients receive the appropriate treatment?
(4) When a non-licensed product is used, are data that can be used to help with its evaluation collected?
(5) Is there a system for recording adverse reactions and are these appropriately reported nationally?

Declarations of interest

All Working Party members made a declaration of interest. Consultancy, or lecture fees or support to attend scientific meetings had been received by eight individuals from between two and ten product manufacturers. Departmental funding for clinical trials or other research activities had been received from a total of seven different pharmaceutical firms. UKHCDO has received donations from eight manufacturers or distributors of products used in the treatment of haemophilia.

Working committee

Christopher Ludlam (Chariman), David Keeling (Secretary), Trevor Barrowcliffe (National Institute for Biological Standards and Control), Elizabeth Chalmers, Paul Giangrande, Christine Harrington (Haemophilia Alliance and UK RCN Haemophilia Nurses Association), Frank Hill, Christopher Hodgson (Haemophilia Alliance and UK Haemophilia Society), Christine Lee, Mike Makris, Henry Watson.

Review of guideline

The guideline is considered at the regular meetings of the UKHCDO Advisory Committee and will be formally reviewed in October 2005.

Appendix 1

Factor VIII concentrates

Recombinant FVIII products

Kogenate (Bayer) and Helixate NexGen (Aventis Behring) These are identical products, manufactured in a single plant in the US (Berkeley, CA), but distributed under different brand names by the two companies. The gene for FVIII has been inserted into an established cell line from baby hamster kidney (BHK). The secreted recombinant FVIII is processed by multiple purification steps, including ion-exchange chromatography gel filtration/size exclusion chromatography and double immunoaffinity chromatography using a murine monoclonal antibody. These second-generation products now include a specific virucidal inactivation step (solvent/detergent treatment) during the manufacture. In contrast to the original version, the product is stabilized in the final formulation with sucrose. Although albumin is no longer added to the final vial, it is present in trace quantities in the product as it is a component of the cell culture medium.

Recombinate (Baxter) Manufactured in two plants, one in Europe and the other in the USA (Neuchatel, Switzerland and Los Angeles, CA, USA). The genes for FVIII and vWF have been inserted into CHO cells. The vWF acts as a stabilizer for FVIII in cell culture but no vWF is actually present in the final formulation. The culture medium contains added bovine proteins. The recombinant FVIII produced is extracted from the culture medium and purified by single immunoaffinity chromatography using a murine monoclonal antibody. It is separated from vWF by elution in the presence of calcium chloride. After further concentration using ultrafiltration and additional purification with Sepharose chromatography, the final product is lyophilized and stabilized by the addition of pasteurized human albumin. A new version of this product in which albumin has been entirely removed is currently undergoing clinical trial.

ReFacto (Wyeth-Genetics Institute) Manufactured in two plants in Europe and the USA (Stockholm, Denmark and St Louis, MI, USA). This product is manufactured from a CHO cell line, in which a modified human FVIII gene has been inserted using a vector. The modified gene, which encodes a single-chain 170-kDa polypeptide, was derived from full-length cDNA by removing the major part of the region encoding the B-domain. The CHO cells are cultured in a serum-free medium. The purification process comprises five different chromatography steps including immunoaffinity with monoclonal antibodies directed to the heavy chain of FVIII and a chemical solvent/detergent virus inactivation step. Although albumin is not added to the final formulation, the product does contain traces of human plasma-derived albumin, as this is a component of

the cell culture medium. It is important to use the specific concentrate standard (provided by the company) when carrying out post-infusion assays.

Plasma-derived FVIII concentrates

8Y (BPL) This FVIII concentrate is now manufactured in Elstree (UK) using plasma imported from the USA. After initial cryoprecipitation, contaminant fibrinogen and fibronectin are removed by precipitation with heparin. FVIII is then precipitated with a mixture of glycine and sodium chloride and lyophilized. Dry superheating is then applied at 80 °C for 72 h. This concentrate contains therapeutic quantities of vWF.

Alphanate (Alpha) Manufactured in the USA (Los Angeles, CA) using plasma from apheresis donors. The cryoprecipitate solution is subjected to polyethylene glycol (PEG) precipitation to purify the antihaemophilic fraction, which is then purified using heparin ligand chromatography. It is subjected to two viral inactivation steps: solvent/detergent treatment (using a combination of TNBP and polysorbate-80) and final heat treatment at 80 °C for 72 h following lyophilization. This concentrate contains therapeutic quantities of vWF.

Beriate P (Aventis Behring) Manufactured in Marburg, Germany, using plasma derived from donors in Austria, Germany and the USA. Cryoprecipitate solution is subjected to purification with ion-exchange chromatography. The product is subjected to pasteurization in aqueous solution at 60 °C for 10 h. No albumin is added as a stabilizer and the product is stabilized in amino acids and saccharose. This concentrate is not suitable for the treatment of vWD.

Fanhdi (Grifols) Manufactured in Barcelona (Spain) using imported plasma derived from USA donors. The cryoprecipitate solution is subjected to PEG precipitation to purify the antihaemophilic fraction, which is then purified using heparin ligand chromatography. It is subjected to two viral inactivation steps: solvent/detergent treatment (using a combination of TNBP and polysorbate-80) and final heat treatment at 80 °C for 72 h following lyophilization. This concentrate contains therapeutic quantities of vWF.

Hemofil-M (Baxter) Manufactured in the USA, using plasma from apheresis donors. Although the plasma source is not the same, the manufacturing process is essentially the same as that used to manufacture Replenate. Resuspended cryoprecipitate is cooled and the resultant precipitate, mostly fibrinogen and fibronectin, is removed by centrifugation. Viral inactivation is performed using a solvent/detergent combination of TNBP and triton X-100. Following column chromatography with Sepharose bound anti-FVIII:C monoclonal antibody, the FVIII is eluted with ethylene glycol. The effluent is further purified by ion-exchange chromatography and lyophilized. This product contains no vWF.

Liberate (SNBTS) Manufactured in Edinburgh, UK, using plasma derived from donors in the USA and Germany. Cryoprecipitate is washed and resuspended and the concentrations of fibrinogen and fibronectin are reduced by further precipitation. After adsorption with aluminium hydroxide to remove residual prothrombin complex, the FVIII undergoes solvent/detergent treatment (with TNBP and polysorbate-80) and ion-exchange chromatography. A new version (Liberate HT), which incorporates an additional heat-treatment step (80 °C for 72 h), is undergoing clinical trial.

Monoclate-P (Aventis Behring) Manufactured in the USA from American apheresis donors. The cryoprecipitate is dissolved in a water–alcohol mixture in order to precipitate fibrinogen and cold insoluble globulins. Prothrombin complex factors are removed by adsorption with aluminium hydroxide. The cryosolution is then concentrated and dialysed to remove alcohol and stabilizers are added prior to pasteurization in solution at 60 °C for 10 h. The diluted pasteurized solution is passed through an immunoaffinity resin column with a solid-phase murine monoclonal antibody to vWF. The column is washed and the FVIII is separated. This product contains no vWF.

Replenate (BPL) Manufactured in Elstree (UK) using imported American plasma. The method used for manufacture is essentially that to make Hemofil-M. Resuspended cryoprecipitate is cooled and the resultant precipitate, mostly fibrinogen and fibronectin, is removed by centrifugation. Viral inactivation is performed using a solvent/detergent combination of TNBP and Triton X-100. Following column chromatography with sepharose bound anti-FVIIIC monoclonal antibody, the FVIII is eluted with ethylene glycol. The effluent is further purified by ion-exchange chromatography and lyophilized. This product contains no vWF.

Factor IX concentrates

Recombinant FIX

Recombinant FIX (Benefix) (Baxter) Recombinant FIX is manufactured in a single plant in the USA by Wyeth-Genetics Institute (Cambridge, MA), although the product is marketed by Baxter in Europe. Recombinant human FIX is expressed by CHO cells and purified using four sequential chromatographic steps and a final viral retention filtration step. The CHO cells are grown and produce recombinant FIX in a defined, serum-free medium lacking any added protein components. Following purification, the recombinant FIX is diafiltered into an albumin-free formulation and lyophilized.

Plasma-derived FIX concentrates

Alphanine (Alpha) This concentrate is manufactured in the USA (Los Angeles, CA) using plasma from apheresis donors. Cryoprecipitate supernatant is adsorbed using DEAE and then purified using ion-exchange and carbohydrate ligand (dual polysaccharide affinity) chromatography steps. It is subjected to solvent/detergent virus inactivation treatment with a combination of (TNBP/polysorbate-80) and to an in-process 15-nm nanofiltration virus removal step. The FIX is formulated with heparin and dextrose and lyophilized.

HIPFIX (SNBTS) This is manufactured in Edinburgh, UK, using plasma imported from the USA and Germany by ion-exchange chromatography from cryoprecipitate supernatant. After two ion-exchange purification steps, it undergoes solvent/detergent treatment (with a combination of TNBP/polysorbate-80) and heparin affinity chromatography. A small amount of antithrombin is also added. After lyophilization it is subjected to dry heat treatment at 80 °C for 72 h.

Mononine (Aventis Behring) Manufactured in Kankakee (USA) using plasma from apheresis donors in the USA. Prothrombin complex concentrate is made by standard methodology using ion-exchange DEAE chromatography and applied to an immunoaffinity chromatography column containing a monoclonal anti-FIX antibody. The FIX is eluted using sodium thiocyanate, which is removed by diafiltration. Dual ultrafiltration allows the passage of FIX whilst retaining viruses. The product is then concentrated and aminohexyl-sepharose gel is added in order to remove trace residual murine antibodies. After elution from the gel, the FIX is lyophilized.

Replenine VF (BPL) Manufactured in Elstree (UK) from imported American (apheresis-derived) plasma. Cryoprecipitate supernatant is adsorbed to an ion-exchange gel, washed with a low-salt buffer, and FIX eluted with a buffer containing a higher concentration of sodium chloride. After viral inactivation by TNBP and Tween-80, the FIX is further purified by metal chelate (copper-Sepharose) chromatography, which allows removal of other contaminating proteins. The final product is subjected to 15-nm nanofiltration and the product is lyophilized in the final formulation in the presence of glycine.

Products for the treatment of patients with inhibitors

FVIII and FIX concentrates are used to treat patients with haemophilia A and B complicated by inhibitors. Other treatments are:

Porcine FVIII (Hyate:C, Ipsen)

Porcine FVIII is manufactured in Wrexham (UK) using plasma obtained from British pigs. The plasma is fractionated through conventional cryoprecipitation and purified using polyelectrolyte ion-exchange chromatography. It is not subjected to any specific virucidal treatment. Porcine VIII is not generally available for reasons discussed under the section 'Transfusion Transmitted Infection'.

FEIBA (Baxter)

FEIBA is a plasma-derived prothrombin-complex concentrate. It is manufactured in Vienna (Austria) using plasma obtained from apheresis donors from several countries including the USA, Austria, Germany and Sweden. This product is prepared from cryoprecipitate supernatant, which then undergoes a process of controlled surface activation. After a series of purifying, adsorption and filtration steps the product is vapour heated initially at 60 °C for 10 h and subsequently for a further 1 h at 80 °C under increased atmospheric pressure (375 mbar) before lyophilization.

Recombinant factor VIIa (NovoSeven, Novo Nordisk)

This recombinant product is manufactured in a single plant near Copenhagen (Denmark). FVII is

produced as a single-chain glycoprotein (406 amino acids, 50 kDa), in a genetically transformed BHK cell line. Purification is by ion-exchange and immunoaffinity chromatography using murine monoclonal antibodies. During purification, recombinant FVII is converted to the two-chain activated form. The manufacturing step includes a nanofiltration step for viral elimination. The recombinant VIIa is formulated as a freeze-dried preparation in the final vial, and no albumin is added. The recombinant VIIa contains non-coagulation factor contaminants as a result of the manufacturing process. These include trace amounts of hamster proteins from cells used in the fermentation process, bovine IgG and bovine serum in the fermentation medium.

Concentrates for the treatment of von Willebrand desease

8Y (BPL), Alphanate (Alpha) and Fanhdi (Grifols)

These products have been discussed earlier.

Haemate P (Aventis Behring)

Manufactured in Marburg (Germany) using plasma derived from donors in Germany, Austria and the USA. The source cryoprecipitate undergoes initial adsorption with aluminium hydroxide followed by glycine precipitation, which removes fibrinogen in the precipitate. Subsequent treatment of the glycine supernatant with sodium chloride concentrates and precipitates the FVIII. This is pasteurized at 60 °C for 10 h in the presence of stabilizers prior to a second sodium chloride precipitation. Albumin is added before lyophilization.

vWF concentrate (LFB)

Manufactured in Lille (France) using plasma obtained from French donors, this product differs from all the others in this category in that it does not also contain therapeutic quantities of FVIII. After adsorption using aluminium hydroxide, the vWF is extracted and purified using anion-exchange and affinity chromatography. It is subjected to solvent/detergent treatment (using a combination of TNBP/polysorbate-80) as a virucidal step. A new version of this product is currently undergoing clinical trials. This incorporates three viral elimination steps (solvent/detergent treatment, dry-heat treatment, nanofiltration).

Products for other congenital bleeding disorders

Fibrinogen concentrates

Fibrinogen concentrate (SNBTS) Imported plasma from the USA and Germany is subjected to initial cryoprecipitation, followed by ion-exchange chromatography. It is subjected to two virucidal steps, including an initial solvent/detergent treatment (using a combination of TNBP/polysorbate) and after lyophilization it is dry heated at 80 °C for 72 h.

Haemocomplettan HS (Aventis Behring) This product is manufactured in Marburg (Germany) using plasma obtained from donors in the USA, Germany and Austria. The fibrinogen is prepared from the glycine supernatant of the intermediate-purity FVIII process. The final product is a purified concentrate of fibrinogen pasteurized at 60 °C for 10 h.

Factor VII

Factor VII (BPL) Manufactured in the UK using imported plasma obtained from apheresis donors in the USA. It is prepared using ion-exchange chromatography and the final product has a specific activity of 1.5–2 units mg^{-1} protein. It is subjected to dry heat treatment at 80 °C for 72 h. It has a relatively short shelf life compared to other coagulation factor concentrates.

Factor VII (Baxter) Manufactured in the former Immuno plant in Austria using plasma derived from several countries, including the USA, Austria and Germany. It is prepared using ion-exchange chromatography. The product is vapour heated initially at 60 °C for 10 h and subsequently for a further 1 h at 80 °C under increased atmospheric pressure (375 mbar) before lyophilization.

Factor VII-LFB (LFB) Manufactured in France using plasma derived from French donors. It is prepared using initial DEAE adsorption followed by anion-exchange chromatography. The concentrate is subjected to solvent/detergent treatment (using a combination of TNBP and polysorbate-80). The final product has a specific activity of 1–2 units mg^{-1} protein.

Recombinant factor VIIa (NovoSeven) This product has been used for the treatment of congenital FVII deficiency. Although there are limited data that suggest that this product is effective, it is not licensed for this indication.

Factor XI

Factor XI (BPL) Manufactured in Elstree (UK) from imported American US plasma. It is prepared from cryoprecipitate supernatant, and FXI co-fractionates with antithrombin on heparin-Sepharose. The FXI-rich fraction is formulated, lyophilized and dry-heated at 80 °C for 72 h. Heparin has been added to reduce thrombogenicity.

Hemoleven (LFB, France) Manufactured in Lille using plasma from French donors. It is only available on a named patient basis. It is subjected to two separate elimination steps, including solvent/detergent treatment and 15-nm nanofiltration. In view of the reported risk of thrombogenicity with other FXI concentrates, small quantities of heparin, antithrombin and C-1 esterase inhibitor are added to the product.

Factor XIII The only preparation now available in the UK is Fibrogammin HS (Aventis Behring). This is derived from plasma from donors in the USA, Germany and Austria. It is produced by conventional cryoprecipitation followed by purification with ion-exchange chromatography. Albumin is added to the preparation as a stabilizer. The final product is subjected to pasteurization (60 °C for 10 h).

Prothrombin complex concentrates

These generally contain therapeutic quantities of FII (prothrombin), FIX and FX. They may be used in the treatment of congenital deficiency of FII and FX, in the absence of specific concentrates. They offer the advantage over plasma of being subjected to virucidal treatment and they contain known quantities of the relevant coagulation factor. The potency of the vial is usually expressed in terms of the FIX content.

Beriplex PN (Aventis Behring) Manufactured in Germany from plasma from donors in Germany, Austria and the USA. The cryoprecipitate supernatant is adsorbed with aluminium hydroxide, and fractionated using DEAE–Sephadex. The eluate contains FII, FVII, FIX and FX and is pasteurized (60 °C for 10 h) prior to nanofiltration as an additional virus inactivation step. Albumin, heparin and antithrombin are also added.

HT DEFIX (SNBTS) Manufactured in Edinburgh, UK, from plasma obtained from donors in Germany and the USA. DEFIX contains FII, FIX and FX but not FVII. It is prepared from cryoprecipitate supernatant by anion-exchange chromatography using fractions whose low thrombogenicity potential have been selected by *in vitro* tests. Antithrombin is also added to the product. After lyophilization it is heated at 80 °C for 72 h.

Profilnine SD (Alpha) Manufactured in the USA (Los Angeles, CA) using plasma from apheresis donors from cryoprecipitate supernatant by DEAE–cellulose adsorption. It is subjected to a solvent/detergent virus inactivation treatment (TNBP/polysorbate-80). The product contains FII, FIX and FX but only low levels of FVII.

Prothromplex (BAXTER) Manufactured in Austria, using plasma obtained from apheresis donors from several countries including the USA, Austria, Germany and Sweden. It is prepared from cryoprecipitate supernatant from which FII, FIX and FX are adsorbed on to DEAE–Sephadex. The eluate is further processed and the product is vapour heated initially at 60 °C for 10 h and subsequently for a further 1 h at 80 °C under increased atmospheric pressure (375 mbar) before lyophilization. Antithrombin and heparin are also added to the product. Prothromplex T (Baxter) is a similar product but contains FVII as well.

Fresh frozen plasma

FFP is a source of all coagulation factors but also contains isoagglutinins and needs to be administered with regard to the patient's blood group. UKHCDO has already recommended the preferential use of FFP subjected to a virucidal treatment step. The only such preparation currently licensed in the UK is Octaplas (Octapharma), a commercial preparation of pooled plasma (derived from donors in the USA, Germany and Austria) and subjected to treatment with TNBP and Triton X-100. However, the National Blood Authority and Scottish National Blood Transfusion Service (SNBTS) have recently introduced packs of FFP derived from single donors that have been subjected to treatment with methylene blue, which is subsequently removed by filtration. All FFP currently supplied by the National Blood Authority and SNBTS is derived from UK volunteer donors. It will shortly be imported from countries where BSE and vCJD are not prevalent for use in children born after 31 December 1995.

Cryoprecipitate It is prepared from FFP by slow thawing at 4 °C for 24 h. Cryoprecipitate appears as an insoluble precipitate and is separated by

centrifugation. It contains significant quantities of FVIII, vWF, fibrinogen and FXIII (but not FIX or FXI). The coagulation factor content of individual packs is variable and not controlled. Furthermore, cryoprecipitate is not subjected to viral inactivation procedures (such as heat or solvent/detergent treatment).

Appendix 2

Other therapeutic agents

Desmopressin (DDAVP)

Desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) is a synthetic analogue of the nonapeptide arginine vasopressin. The administration of DDAVP can significantly boost levels of FVIII and vWF in the blood and it is, therefore, a valuable agent for the treatment of mild haemophilia A and vWD. It is also very useful in the treatment of carriers of haemophilia A, many of whom have significant reductions in the baseline level of FVIII. In contrast, the administration of DDAVP has no effect on the FIX level, and is thus of no value in haemophilia B (Christmas disease).

Intravenous injection is the most common route: the standard dose of DDAVP for treatment of bleeding disorders is 0.3 μg kg^{-1}, diluted in physiological saline and administered by slow intravenous infusion. The side-effects of DDAVP include headache, tachycardia, facial flushing, abdominal pain, tremor and sweating during or shortly after intravenous administration. These symptoms are quite common but are usually simply the consequence of rapid intravenous infusion and symptoms usually quickly subside after slowing, or even temporarily stopping, the infusion. Subcutaneous administration using a more potent formulation (Octim 15 μg mL^{-1}) is being increasingly adopted. A concentrated nasal spray preparation (Octostim 150 μg per dose) has also proved to be effective for home treatment of patients with bleeding episodes and it can also be used prophylactically, although it is available only on a named patient basis at present. It is important to appreciate that the nasal spray preparation (Desmospray) used for the treatment of diabetes insipidus is too dilute for use in disorders of haemostasis.

DDAVP should be avoided in patients with heart failure or other conditions being treated with diuretic agents. The data sheet gives unstable angina as a contra-indication but in view of anecdotal reports of myocardial infarction and cerebral infarction it is best avoided in all those with known atherosclerosis. Care should always be taken after use of DDAVP to prevent fluid overload and consequent hyponatraemia, especially so when repeated doses are used. Weighing the patient and measuring the plasma sodium during prolonged administration can readily monitor this. Great care must be taken with peri- and post-operative intravenous fluids. Some prefer not to use DDAVP in those less than 2 years old because of the risk of hyponatraemia and seizures, which is increased in infants and young children. If it is used in this age group, it should be used with caution and then with close surveillance, fluid restriction, avoidance of hyponatraemic solutions, and close monitoring of serum electrolytes and urine output for at least 24 h after administration. DDAVP is not contra-indicated in uncomplicated pregnancy though like all drugs it should be used with caution. No teratogenic effect has been observed in animals and its prolonged use (at lower dosage) in diabetes insipidus has shown no adverse effects for mother or fetus.

Tranexamic acid

Tranexamic acid (Cyklokapron®) is a structural analogue of lysine, which binds irreversibly to the lysine-binding sites on plasminogen, thus inhibiting binding to fibrin and thus the whole process of fibrinolysis. It inhibits the natural degradation of fibrin and helps to stabilize clots. In elective procedures it is, therefore, likely to be more effective if given beforehand so that it is circulating when fibrin is formed. The plasma half-life is 2 h.

Antifibrinolytic agents are of particular benefit in the treatment of bleeding from the gastrointestinal tract, menorrhagia, epistaxis and oral bleeding (including dental surgery) in patients with congenital and acquired coagulation disorders. It can be given orally (15–25 mg kg^{-1} tds) or intravenously (10 mg kg^{-1} tds). Alternatively, it can be given as a mouthwash (10 mL of a 5% solution four times a day). The dose must be reduced in patients with renal failure or impairment.

Tranexamic acid should not be used in association with FEIBA or other prothrombin complex concentrates because of the potential for thrombogenicity. In contrast, administration of tranexamic acid may enhance the efficacy of recombinant FVIIa (NovoSeven). Tranexamic acid should not to be used to treat haematuria because of blood loss from the upper urinary tract as this can provoke painful clot retention and even renal failure associated with bilateral ureteric obstruction.

Side-effects of tranexamic acid are rare and mainly limited to nausea, diarrhoea or abdominal pain. These symptoms are usually associated with high doses, and usually subside if the dose is reduced. Hypotension is occasionally observed, typically after rapid intravenous infusion.

Fibrin sealants

Beriplast P Combiset (Aventis Behring) This fibrin sealant kit consists of a vial of human fibrinogen plus human FXIII connected to a diluent vial containing bovine aprotinin solution and a second vial of human thrombin connected to a diluent vial containing calcium chloride solution. The plasma components are pasteurized at 60 °C for 10 h. Aprotinin of bovine origin and calcium chloride solutions are also included. This product does not have a licence.

Fibrin Sealant Kit (SNBTS) Fibrin Sealant is a multi-component product comprising vials of human fibrinogen, human thrombin, calcium chloride and Tris. The human fibrinogen is a freeze-dried preparation that also contains FXIII and fibronectin. This component is manufactured from cryoprecipitate and is subjected to solvent/detergent treatment and a terminal, dry heat treatment at 80 °C for 72 h. Human thrombin is a freeze-dried preparation manufactured from cryoprecipitate supernatant plasma by ion-exchange chromatography and is also subjected to solvent/detergent and dry heat treatment at 80 °C for 72 h. This product does not have a licence.

Tisseel (Baxter) Tisseel concentrate contains human fibrinogen, fibronectin, FXIII and plasminogen. Two concentrations of human thrombin are included in the kit to allow for either rapid (within 4 s) or delayed (up to 60 s) setting of the sealant dependent on the type of operative procedure being performed. The kit contains bovine aprotinin. Both the Tisseel and human thrombin components are subjected to vapour heating. This product has a licence.

Quixil (Omrix) This is supplied as a kit containing two components: human thrombin and lyophilized cryoprecipitate rich in both fibrinogen and FXIII. The latter also contains tranexamic acid as a clot stabilizer. The kit is also supplied with a double-barrelled syringe, so that it can be sprayed on to a surgical area. It is only licensed for use in the setting of liver surgery. In view of reported adverse reactions, it must not be used in any surgical procedure where it could come into contact with the CSF or dura mater. This product has a licence.

Appendix 3

Level	Type of evidence (based on AHCPR)
Ia	Evidence obtained from meta-analysis of randomized controlled trials
Ib	Evidence obtained from at least one randomized controlled trial
IIa	Evidence obtained from at least one well-designed controlled study without randomization
IIb	Evidence obtained from at least one other well-designed quasi-experimental study
III	Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case–control studies
IV	Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities

Grade	Recommendation (based on AHCPR)
A (evidence levels Ia, Ib)	Requires at least one randomized controlled trial as part of the body of literature of overall good quality and consistency addressing the specific recommendation
B (evidence levels IIa, IIb and III)	Requires availability of well-conducted clinical studies but no randomized clinical trials on the topic of recommendation
C (evidence level IV)	Requires evidence from expert committee reports or opinions and/or clinical experience of respected authorities. Indicates absence of directly applicable studies of good quality

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