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Factors affecting the quality, safety and marketing approval of clotting factor concentrates for haemophilia

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Abstract

Selecting therapeutic products for the treatment of haemophilia follows the process of obtaining market approval of products submitted to the scrutiny of a regulatory agency. In well-resourced countries, key decisions on whether a product is sufficiently safe and of high quality are made by highly expert and well-resourced agencies, such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). In countries lacking such agencies, well-informed decisions can still be made through an appreciation of the key issues affecting the quality, safety and efficacy of haemophilia products. A number of well-established principles may then be applied in order to make a choice. In this review, reflecting principles outlined by the World Federation of Hemophilia, we outline the key features in determining the acceptability of therapeutic products for haemophilia in order to ensure an optimal choice in all the environments providing haemophilia care.

Keywords: haemophilia, therapeutic products, safety.

Introduction

Selecting therapeutic products for the treatment of haemophilia follows the process of obtaining market approval for products submitted to the scrutiny of a regulatory agency. In well-resourced countries, key decisions on whether a product is sufficiently safe and of high quality are made by highly expert and well-resourced agencies, such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). In countries lacking such agencies, well-informed decisions can still be made through an appreciation of the key issues affecting the quality, safety and efficacy of haemophilia products. A number of well-established principles may then be applied in order to make a choice. In this review, reflecting principles outlined by the World Federation of Hemophilia[1], we outline the key features determining the acceptability of therapeutic products for haemophilia in order to ensure an optimal choice in all the environments providing haemophilia care.

Features affecting the safety and efficacy of haemophilia treatment concentrates

Pathogen safety

Since the 1980s, manufacturers and the agencies regulating the manufacture of fractionated plasma products have responded to concerns about transmission of blood-borne viruses by developing a comprehensive set of measures designed to reduce, if not eliminate, infectious risk. These measures are based on the following principles:

1) selection of appropriate blood and plasma donors;
2) screening of the plasma raw material with laboratory tests;
3) elimination of any contaminating viruses through the manufacturing process;

Specifically, measures to enhance the pathogen safety of plasma products include:

- selection procedures that ensure the exclusion of donors with high-risk behaviour;
- mandatory serological testing on all plasma donations for the main transfusion-transmitted viruses: human immunodeficiency virus (HIV), hepatitis B (HBV), and hepatitis C (HCV);
- plasma inventory hold and exclusion based on post-donation information;
- nucleic acid testing (NAT) of minipools for HCV, HIV, HBV, human parvovirus B19, and hepatitis A virus (HAV), and exclusion of reactive donations;
- testing start-manufacturing plasma pool samples for viral markers and viral genomic material;
- inclusion of one or more validated specific viral inactivation and/or removal steps in the manufacturing process;
- full traceability of plasma from donors to end products.

These principles are also applicable to the products of recombinant manufacture. The relevant cell lines and culture fluids must be selected from low-risk sources, and, when possible, screened for potential pathogenic contaminants.

In particular, pathogen elimination techniques, introduced as steps in the manufacture dedicated to this purpose, are mandatory[2], and these have played the biggest role in improving the safety of haemophilia

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treatment products. This is because, particularly for products made from large numbers of individual plasma donations, the level of clearance achieved through a validated manufacturing process significantly exceeds the level that can be achieved through donor selection and screening procedures[3].

The combination of appropriate donor selection procedures, screening with the current generation of standard serological tests, and, in particular, the inclusion of measures to inactivate or remove viruses, has made fractionated plasma products free from serious known blood-borne viruses such as HIV, HBV, and HCV. In the well-regulated markets of North America and Europe, infections from these agents have not been observed in the haemophilia community for the past 25 years[4]. Some concerns remain regarding the non-enveloped viruses[5] due to the higher level of resistance to pathogen elimination methods of these viruses. Since their introduction in the late 1980s, no recombinant clotting factor concentrate (CFC) has been associated with pathogen transmission.

Features particular to the assessment of plasma-derived clotting factor products

Plasma quality

Factors that have an impact on plasma quality and safety include:

- plasma handling factors, such as separation, storage, and transport, which also depend on the methods used for collecting plasma. This may be recovered from whole blood or obtained by plasmapheresis, which results in source plasma;
- donor epidemiology (e.g., viral infection, prion disease);
- donor selection and testing procedures (including NAT) to reduce the window period for infection with different viruses.

All these factors affect the safety of fractionated plasma products with respect to transmissible infectious agents. They also affect the yield and specific activity of products.

Donor selection

Donor selection procedures are designed to identify and exclude donors at risk of being infected with viruses that can be transmitted by blood transfusion. In developed countries, donor selection procedures have reached a high level of sophistication and complexity, and regulators have included these procedures in their assessment of overall safety of material used to manufacture plasma products[6]. In Europe, directives related to the collection process and donor selection will probably be reconsidered in the next few years as there is room for improvement and there should be an acceptable balance between donor and patient welfare, and also between risk to transfusion safety and risk of compromising the blood supply[7].

Exclusion criteria for donors used in different regulatory climates include a history of:

- blood-borne infections;
- intravenous drug use;
- high-risk sexual behaviour (e.g., men who have sex with men, prostitution);
- receiving human biological materials (e.g., blood, tissue);
- behaviour at risk (e.g., tattoos, piercing);
- medical procedures (e.g., certain illnesses, surgery).

As for all the measures described in this Guide, the ability of different countries to implement these measures may vary. Each regulatory authority must assess a country's local needs before mandating specific measures.

Plasma types

Plasma types may be distinguished according to donor remuneration status (paid or unpaid) and method of collection (recovered or source plasma). Recovered plasma is a by-product of donated whole blood and is generally obtained from unpaid donors. Source plasma is collected from donors, most of whom are paid, through a process known as plasmapheresis that removes only the donor's plasma. The generation of a pool of committed, regular source plasma donors could favour a rapid increase in collection efficiency and should be encouraged[8].

The performance of NAT on plasma for fractionation has greatly reduced the viral load of HIV and HCV from all donor types.

Following selection and screening, the incorporation of viral reduction steps inactivates or removes the residual (low) viral load with equal efficacy for both recovered and source plasma[9]. The introduction of measures by the source plasma industry, such as inventory hold and donor qualification, has made this plasma potentially a safer raw material than plasma recovered from whole blood for which many of these measures are not possible. In particular, emerging infections which are not detectable through selection and screening procedures[10] are still a threat to the safety of transfusion products, although advances in the development of similar pathogen reduction technologies for blood components give cause for hope in this area[11,12].

Donor screening

Individual donations of blood are screened to ensure that blood-borne viruses do not enter the plasma pool. Donor screening is currently available for HBV, HCV,

and HIV. All plasma donations should be tested for these three viruses.

Tests that detect viral infection through the immune response of the donor are limited as there is a window period before the body's immune response generates sufficient levels of the immunological marker. During this period, the donor is infectious but the infection is undetectable. In the case of HBV infection, the serological marker detected in traditional blood screening is an antigen (HbsAg) associated with the virus, rather than an indicator of the immune response; nonetheless there is also a window period for this viral infection. With NAT, this period is shortened by detecting the viral genome, which appears in the blood before the immunological markers. The introduction of NAT has decreased the viral load of plasma pools and therefore increases the margin of safety should viral reduction procedures break down (Table I).

Table I - Donor screening tests for blood-borne viruses.

Test	Recommended	Mandatory*
Anti-HIV	Yes	Yes
Anti-HCV	Yes	Yes
HbsAg (HBV)	Yes	Yes
HIV-RNA (NAT)	Yes	No
HCV-RNA (NAT)	Yes	No
HBV-DNA	Yes	No
Parvovirus B19 DNA	Yes	No
HAV RNA	Yes	No

*While nucleic acid testing (NAT) is not mandatory in many countries, it represents the best blood safety practice and is mandatory in some well-regulated countries (e.g., the US Food and Drug Administration).
DNA: deoxyribonucleic acid; HBV: hepatitis B virus; HbsAg: antigen associated with HBV; HCV: hepatitis C virus; HIV: human immunodeficiency virus; RNA: ribonucleic acid; HAV: hepatitis A virus.

Inventory hold

Inventory hold is the holding of plasma in (frozen) storage before it is processed into concentrates. A plasma donation is held until testing of the donor ensures that the donation was not collected while the donor was in the window period of infection. The use of inventory hold pending qualification of plasma donors further enhances safety and is an attractive, although not mandatory, feature. This measure is particularly useful for source plasma, as plasmapheresis donors can donate more frequently, which may result in more donations during the infectious window period. In the transfusion sector, some countries require the quarantine of plasma destined for transfusion, as a substitute for pathogen reduction[13]. The particular features of an inventory hold vary among organisations. It is most effective when donations that are not re-tested remain unused, whether the donor returns or not.

Viral reduction processes

There are two types of viral reduction processes: 1) inactivation (viral kill); and 2) removal of virus through purification of protein. Viral reduction procedures in the manufacturing process have had the greatest impact on enhancing the safety of haemophilia treatment products. While all the components of the blood safety chain are required for product safety, manufacturing processes can have an especially significant role. For example, solvent-detergent treatment rendered pooled haemophilia treatment products safe from HCV before the introduction of viral testing increased the safety of normal blood transfusions and single-donor cryoprecipitate from HCV transmission[14]. While donor selection and screening of donations, combined with appropriate NAT (and inventory hold where it can be achieved) have significantly reduced the risk of blood-borne viruses entering the fractionation pool, any plasma pool, containing tens of thousands of donations, may contain levels of virus capable of transmitting infection. The inclusion in the fractionation process of one or more steps with validated capability to inactivate and/or remove relevant viruses, primarily enveloped viruses (i.e., HIV, HBV, and HCV), results in plasma products that are essentially free from risk of these viruses. However, current inactivation and removal processes are less effective for non-enveloped viruses (e.g., HAV, parvovirus B19[15]), and this concern can be extended to unknown viruses and infectious agents. Uncertainty remains regarding the transmission of parvovirus B19 *via* concentrates manufactured in the era of NAT viral reduction[15], but this may be due to the use of less well-accredited processes during the earlier years of surveillance[16]. The lack of transmission to haemophilia patients of any of the newly emerging agents challenging the blood safety environment in past decades demonstrates that the processes are robust and can eliminate unknown agents. This situation is in contrast with that of the recipients of transfused components, where these agents, such as West Nile Virus (WNV), Dengue virus, etc., have been transmitted[17,18].

There are a number of different viral reduction methods available, including solvent-detergent, heat treatment (e.g., pasteurisation, dry-heat, steam heat), and nanofiltration. The advantages and limitations of these are outlined in Table II. Relative to the highly pathogenic nature of blood-borne viruses (i.e., HIV, HCV, and HBV), the unbroken safety record of factor concentrates treated with solvent-detergent[14] is a strong argument for making this viral-reducing method a mandatory component in the manufacture of such products.

While nanofiltration is generally very effective for reducing non-enveloped viruses in plasma products, this process is more suitable for the preparation of

Table II - Advantages and points to consider when selecting viral reduction methods for factor concentrates. (Adapted from Burnouf and Radosevich[29]).

Method	Advantages	Points to consider
Solvent-detergent (SD) Treatment with a mixture of chemicals - solvents and detergents - that inactivates viruses through the removal of the lipid envelope that coats some types of viruses. This method is ineffective against non-enveloped viruses	- Extremely efficient against enveloped viruses - Requires relatively simple equipment - Non-denaturing effect on proteins - High recovery of protein functional activity	- Requires a subsequent manufacturing step to eliminate the SD agents - Ineffective against non-enveloped viruses (e.g., HAV, parvovirus B19)
Pasteurisation A generic term for the heat treatment of a protein in solution at 60 °C for 10 hours. Its efficacy in inactivating viruses is dependent on the exact conditions under which it is performed. When it is used on fragile proteins, such as clotting factors, the solution must include protective chemicals to preserve the proteins; however, these chemicals may also preserve viruses. Each process must be evaluated on the basis of the data submitted by the manufacturer	- Potential to inactivate enveloped and non-enveloped viruses, including HAV - Requires relatively simple equipment	- Dependent on conditions - Protein stabilisers may protect viruses - Does not inactivate parvovirus B19 - Low recovery of fragile clotting factors - Potential generation of neoantigens
Vapour-heat Currently restricted to one manufacturer	- May inactivate enveloped and non-enveloped viruses, including HAV	- Possible risk of transmission of HCV and HBV reported - Does not inactivate parvovirus B19
Terminal dry-heat Involves heating the final product in the lyophilised state in the container used to issue and reconstitute the concentrate. The efficacy of viral kill is strongly dependent on the exact combination of time and temperature to which the product is exposed. Conditions described by manufacturers include: - 60 °C for 72 hours - 80 °C for 72 hours - 100 °C for 30 minutes - 100 °C for 120 minutes - 65 °C for 96 hours Each process must be evaluated on the basis of the data submitted by the manufacturer. For example, 60 °C is known to be less effective than 80 °C, when applied for similar lengths of time	- May inactivate enveloped and non-enveloped viruses, including HAV - Treatment applied on the final container	- Does not inactivate parvovirus B19 - Results in 10-20% loss of clotting factor activity - Requires strict control of residual moisture content
Nanofiltration through 15-nm membranes	- Elimination of viruses based on size-exclusion effect - Eliminates all major viruses, including HAV and parvovirus B19 - May eliminate prions - Integrity and removal capacity of the filter can be validated after use - High recovery of protein activity - Non-denaturing for proteins - Risks of downstream contamination are limited when filtration is performed prior to aseptic filling - Filters are commercially available; no royalties	- Not applicable to high molecular weight protein concentrate (without significant protein loss)
Nanofiltration through 35-nm membranes	- Similar to the advantages associated with nanofiltration through 15-nm membranes - Applicable to some FVIII and VWF concentrates	- Incomplete elimination of small viruses

HAV: hepatitis A virus; HCV: hepatitis C virus; HBV: hepatitis B virus; FVIII: Factor VIII; VWF: von Willebrand factor.

FIX concentrates[10]. It should be a mandated feature of concentrates for haemophilia B. Nanofiltration has been successfully applied to FVIII concentrates manipulated to dissociate FVIII from von Willebrand factor (VWF)[19], and to concentrates containing the smaller B-domain-deleted recombinant FVIII molecule[20]. Overall, while non-enveloped viruses continue to pose a greater challenge than enveloped viruses, the technologies in hand, coupled with the diminution in viral load through the application of NAT, have been well validated for the known non-enveloped viruses.

Failures in testing, processing, or critical quality systems are more likely to result in the release of a batch of product with increased risk of infection than any fundamental deficiency in process design or competence. Because of the importance of viral elimination in the ultimate safety of plasma products, there is no room for failure in the steps of the process that are key to viral elimination. Process validation, and the systems at the heart of Good Manufacturing Practice (GMP) traceability, segregation of product manufacturing steps to avoid cross-contamination, training, documentation, change control, and deviation reporting are the keys to the reliable manufacture of safe and effective plasma products.

Importance of geographical source of products

A number of blood-borne viruses reported in recent years have not been evident in haemophilia communities in countries with well-established regulatory systems. These include WNV, Chikungunya, Zika, and other agents transmitted primarily by mosquito bites. In some instances these viruses have been transmitted by blood transfusion, but the viral-inactivation processes used in clotting factor manufacture have eliminated them as a risk for haemophilia patients. However, the situation is different in developing countries. It is clear that the classical transfusion-transmitted viruses are still present in the blood supply of some countries and that the rudimentary haemophilia therapies available, such as plasma and cryoprecipitate, continue to constitute a vehicle of infection for people with haemophilia when not subjected to viral inactivation[21-23]. Similarly, newly emerged agents such as WNV and Zika may well be transmitted to haemophilia patients exposed to non-viral-inactivated components. Some technologies for the viral inactivation of cryoprecipitate have been developed and introduced in emerging countries[24].

Variant Creutzfeldt-Jakob disease (vCJD)

Experiments using various animal species have shown that diseases known as transmissible spongiform encephalopathies (TSEs) are transmissible through blood, plasma, and plasma fractions. Depending on the fraction studied and the manufacturing technique used, these studies showed that much of the infectivity could be removed or cleared from the therapeutic product as a result of the manufacturing process[25,26].

Transmission of TSE through infected blood and blood products has been confirmed in humans. There have been 4 cases of human TSE-variant vCJD in recipients of whole blood or red blood cells from donors who developed the disease[27]. Donor selection measures to exclude people who may have been exposed to the pathogen, primarily through residence in countries of high epidemiological risk, have been introduced and appear to have contained the risk. Such measures need to be carefully considered, and risk benefit principles should be applied in order to ensure continued supply of blood, and the avoidance of other pathogens entering the blood supply through altered donor pool composition[28].

Tests for vCJD show that prions can be detected in the blood of individuals in both the symptomatic and pre-symptomatic phases of the disease[30,31]. Even when these tests become available for mass blood screening, the clearance of infectivity through the manufacturing process will remain the main route for minimising the risk of vCJD from pooled plasma products. The clearance achieved by different processes has probably contributed to the absence of overt vCJD disease in recipients of plasma products. Asymptomatic infection has been shown in a haemophilia A patient receiving a low purity FVIII concentrate, following death from a non-vCJD cause[32]. The manufacturing process of the FVIII concentrate involved is known to have a low capacity for clearance of prions. Post-mortem studies of patients exposed to immunoglobulin products manufactured from similarly infectious plasma[33], and using a process which had a prion clearance capacity considerably higher than the implicated FVIII[34], did not reveal parallel vCJD cases.

Advances in recombinant clotting factor concentrates

The production of recombinant clotting factors (CFC) became possible with the cloning and subsequent expression of functional proteins for both FVIII and FIX. Production of recombinant clotting factors in mammalian cell culture required overcoming significant challenges due to the complex post-translational modifications that are essential to their procoagulant function. Since their introduction, the recombinant versions of both FVIII and FIX have proven to be clinically similar to their plasma-derived counterparts. These recombinant products have gone through three generations since 1992. The first versions were produced in animal or human cell culture and stabilised with human serum albumin. The next generation was produced in animal or human protein

containing cultures but with no albumin added to the formulation. The third generation is completely synthetic and free of all human or animal proteins. In spite of these advances, adherence to GMP remains essential to ensure product safety.

The first haemophilia CFCs from recombinant sources were few in number and priced beyond the reach of most developing countries. As technology has advanced, more products have entered the market and prices have steadily decreased due to competition. Competitive tendering systems developed by several countries have exerted further downward pressure on prices, such that countries including the UK, Australia, and Ireland now pay lower prices for recombinant CFCs than for plasma-derived products. Authorities charged with ensuring availability and access to haemophilia therapies would do well to keep this in mind.

Modified recombinant clotting factor proteins

A number of products have been developed that feature modifications of the clotting factor protein molecule intended to achieve improved therapeutic properties. For example, certain products have been designed to demonstrate modified post-infusion pharmacokinetics, with a half-life that is longer than that of conventional products[35]. These extended half-life (EHL) products offer the theoretical benefits of requiring less frequent infusion, and/or achieving higher clotting factor trough levels through a prophylactic regimen with a comparable infusion frequency to that of conventional products.

This plethora of recombinant factors in the haemophilia treatment landscape, some of which are produced outside the mainstream plasma protein industry, and many of which can be produced in unlimited supplies, has the potential to increase access to care in countries where it is currently significantly limited.

In this context, some important issues must be considered by regulatory and other authorities:

- underlying the distinguishing features of these modified products is their altered pharmacokinetics. Importantly, the pharmacokinetics of conventional FVIII vary significantly between individual patients infused with the same product. This patient-specific inherent variability suggests that a subset of patients may experience the benefits ascribed to the modified EHL recombinant factors when using conventional recombinant or plasma-derived CFCs. One might also expect that the extent to which the modified products EHLs upon infusion might be impacted by patient-specific pharmacokinetics[36].
- The assessment of efficacy of any concentrate is an integral part of its approval process. In order to generate the evidence required by some regulatory agencies, sponsors of new recombinant CFCs now perform randomised studies comparing prophylaxis to on-demand treatment. Some reimbursement schemes in developed countries may also require this evidence. Authorities in developing countries must be aware that these randomised comparisons are often performed in their jurisdictions, and must insist upon measures to protect patients, as stipulated by the Declaration of Helsinki[37].
- Furthermore, if it is necessary to prove efficacy of a product for prophylaxis, then the comparator population should be placed on a prophylaxis regimen with another product, previously approved for prophylaxis, rather than on-demand therapy with the product under review. This avoids the substantial burden associated with on-demand therapy[38].

In addition, in determining the price of new products employed for replacement therapy and characterised by a better pharmacokinetic profile, pharmacokinetic principles could be used to make reliable suggestions about the price of the new product based on the price of the old one. In fact, using pharmacokinetic rules could make the process of drug pricing more rational and more homogeneous across different countries than it is at the moment[39].

The problem of inhibitors in haemophilia

Due to the measures described above, the risk of infection from haemophilia concentrates has faded significantly in well-regulated environments. The issue of the development of inhibitors to infused clotting factor has now come to the forefront. This adverse event has existed for as long as replacement factor has been used in the management of haemophilia. Inhibitors (antibodies to replacement FVIII or FIX) are a well-characterised immunological response influenced by a number of intrinsic (patient-related) factors such as the presence of specific genetic mutations, a family history of inhibitors, or a specific ethnic background. Inhibitors occur often in previously untreated patients (PUPs) treated with any FVIII product.

Much discussion has been devoted to the question of whether particular factor concentrates are associated with a greater risk of developing inhibitors, and a comprehensive discussion is also ongoing on the data that are emerging from recent studies and international registries[40].

An enhanced risk has been established for plasma-derived FVIII concentrates subjected to certain viral-inactivation procedures that use specific heat treatments[41-43]. This finding prompted examination of the possibility that manufacturing processes can lead to molecular changes and neoantigenicity, or the

development of new epitopes, in the FVIII molecule[44].

The increasing use of recombinant products has led to investigation into whether aspects of their production might enhance the risk of inhibitor development. Some recombinant clotting factors, produced in rodent cell lines, differ from the protein found naturally in humans in several aspects of their glycosylation and other post-translational modifications[45]. The possible effect of these differences on product immunogenicity is surrounded in controversy, particularly in PUPs whose likelihood of developing inhibitors is largely impacted by the multiple intrinsic factors mentioned above. Regulatory authorities consider that the neoantigenicity of factor concentrates is best assessed in previously treated patients (PTPs)[46].

Following several inconclusive observational studies using meta-analyses[47,48], the Survey of Inhibitors in Plasma-Products-Exposed Toddlers (SIPPET)[49] randomised haemophilia A PUPs, or minimally exposed patients, to receive plasma-derived FVIII concentrates containing VWF or recombinant FVIII concentrates, and assessed for inhibitors after 50 days of exposure. A number of different products were used in each arm; all plasma-derived CFCs contained VWF, all recombinant products were raised in rodent cell lines, and none were of the newer EHL type. The study concluded that PUPs treated with the recombinant products had a higher incidence of inhibitors than PUPs treated with the plasma-derived products[50], and that this resulted in plasma products providing a more cost-effective treatment[51]. The Committee for Medical Products for Human Use (CHMP) of the EMA has concluded, however, that there is no clear and consistent evidence of a difference in the incidence of inhibitor development between the two classes of FVIII medicines used in the trial[52], recommending that the evaluation of the risk of inhibitor development should be at the product level instead of at the class level. While acknowledging the basis of the EMA's position, this review considers that the catastrophic effect of inhibitor formation on the well-being of patients with haemophilia demands the application of the precautionary principle in this area. Given that the best available evidence indicates that the risk is significantly reduced if the first exposure to concentrate is through plasma-derived products, it is advisable that these products are used preferentially, at least for the first fifty days of exposure. Subsequently, patients (and their families) and treating physicians may elect to switch to recombinant products. This review considers such an approach optimal from the aspects of safety, and also supply, given that the use of plasma-derived products can accommodate the needs of PUPs, who are generally of low body weight, and there will, therefore, be no problems regarding product access. This review acknowledges that recombinant products, and other non-factor based modalities, are crucial if the treatment of haemophilia is to encompass the global patient population.

The treatment of patients with established inhibitors involves bypassing agents, currently limited to one plasma-derived and one recombinant product with a broadly equivalent therapeutic effect[53], although short-term episodic prophylaxis with recombinant Factor VIIa (rFVIIa) may be an alternative treatment option to on-demand treatment for patients with inhibitors[54]. Additionally, the availability of recombinant porcine factor VIII has roused renewed interest in the use of this modality in the clinical management of these patients[55].

The optimal therapeutic path for patients with inhibitors is removing the need for the inhibitor through tolerisation with clotting factor, a hugely expensive but, ultimately, cost-effective process[56]. The type of product (plasma-derived or recombinant) appears to have no significant effect on the achievement of tolerisation[57].

Conclusions

Since the 1980s, various measures have been introduced to reduce the risk of viral transmission through fractionated plasma products. Not all practices are considered mandatory standards by regulatory agencies, and their use by fractionators must be assessed in the overall context of safety, availability, and cost. Thus, while donor selection can offer significant benefits, other practices, such as NAT to narrow the window period and inventory hold, also reduce the risk of infectious units being pooled. Emerging technologies including viral metagenomics have also been applied to blood donor screening[58], demonstrating the benefit of high-throughput sequencing in the detection of viral sequences of unknown, unsuspected or emerging viruses that may be transmitted to recipients by blood-derived products[59-61].

Some measures may have only limited benefits for users of haemophilia treatment products, while possibly having a negative effect on the yield and financial viability of fractionation processes. For example, limiting donor pool size can reduce the risk of viral transmission, but probably only for infrequent users of plasma products[62].

Donor selection procedures that exclude high-risk donors and serological screening of plasma donations are the mainstay of ensuring safe raw material for the fractionation process. However, in-process inactivation has had the most profound impact on the safety of fractionated plasma products. Even allowing for limited effectiveness against non-lipid enveloped viruses (for which NAT may be used to limit plasma pool viral burden), in-process viral inactivation or removal has reduced the risk of receiving an infected product to an

extremely low level. Establishment and maintenance of GMP and licence-compliant (i.e., validated) conditions are critical to eliminating these areas of risk.

Clinical trials of new treatment avenues, such as bispecific antibodies that replace the function of activated FVIII in the clotting cascade[63], or RNAi-mediated knockdown of anticoagulant activity[64], have extended the therapeutic landscape and may soon provide alternative approaches to the management of haemophilia, especially in the presence of inhibitors. The evaluation of such products would involve a different set of criteria, as they are not based upon replacing the deficient factor (VIII or IX). Furthermore, the dream of gene therapy in haemophilia is also rapidly coming to reality[65].

In the meantime, those authorities responsible for ensuring that haemophilia treatment products are safe and efficacious may find this summary useful:

- fractionated plasma products have a history of transmitting blood-borne viruses (e.g., HBV, HCV, and HIV);
- plasma products manufactured under current best practices, and manufactured with attention to GMP, rank among the lowest risk therapeutic products in use today;
- product safety is the result of efforts in several areas;
- improved donor selection (exclusion of at-risk donors);
- improved screening tests of donations (including NAT);
- type and number of in-process viral inactivation and/or removal steps.

Of these, in-process viral inactivation is the single largest contributor to product safety.

Plasma types are distinguished on the basis of:

- donor remuneration status (paid or unpaid);
- method of collection. In practice, all collection methods yield safe, effective products if processes are properly optimised and GMP is observed.

The inclusion in the fractionation process of one or more steps with validated capability to inactivate or remove relevant viruses, primarily enveloped viruses (e.g., HIV, HBV, and HCV), results in plasma products that are essentially free from risk of these viruses. Inactivation and removal processes are less effective for non-enveloped viruses (e.g., HAV and parvovirus B19).

The demonstration that vCJD can infect people with haemophilia through the infusion of replacement factor products means that manufacturing steps validated for prion clearance must be included in the production of all CFCs.

Assessment of prophylaxis should be performed in head-to-head randomised comparisons with CFCs already approved for this purpose, and should not include the randomisation of patients to on-demand treatment arms.

Inhibitors are a known risk with any CFC and inhibitors occur often in PUPs treated with any FVIII product. Inhibitor development is caused by many risk factors not related to product type. Research in this area is ongoing. Following a thorough review of the SIPPET trial data and other relevant studies, including interventional clinical trials and observational studies, the EMA's CHMP stated in September 2017 that the Pharmacovigilance Risk Assessment Committee (PRAC) concludes there is no clear and consistent evidence of a difference in the incidence of inhibitor development between the two classes of Factor VIII medicines: those derived from plasma and those made by recombinant DNA technology[42]. The seriousness of this adverse effect requires a cautious approach to product choice, and the use of plasma-derived factors for the first fifty days of exposure to treatment should be actively considered.

In the absence of evidence that switching products elicits inhibitors, treating physicians may elect to treat PUPs with plasma-derived FVIII concentrate containing VWF, if it is considered that a high risk for inhibitor development justifies a precautionary approach. These patients may be transitioned to other CFCs following 50 days of exposure to plasma-derived FVIII concentrate containing VWF. PTPs may be safely treated with either type of product. If a plasma-derived FVIII concentrate containing VWF is not available, treatment with a recombinant FVIII concentrate or a plasma-derived FVIII concentrate lacking VWF, that meet the requirements outlined in this guide, remains far superior to witholding treatment.

Patients with inhibitors may be treated with either plasma-derived or recombinant bypassing agents, and, preferably, tolerised with a CFC, also either recombinant or plasma-derived.

Disclosure of conflicts of interest

GML is the Editor-in-Chief of Blood Transfusion and this manuscript has undergone additional external review as a result. AF and JC provide compensated consultancy services to manufacturers of haemophilia treatments. The other Authors declared that they have no conflicts of interest.

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