

## EDUCATION SESSION 2: HAEMOPHILIA



### Update on Clotting Factor Concentrates

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We need concentrates that do not transmit viral infection (a goal now met); that are available in a reliable and adequate supply (a goal soon to be met); that are priced at a reasonable level for less affluent countries (a goal yet to be met); and that meet the needs of patients with rare factor deficiencies (another difficult goal). A great array of concentrates of factor VIII and, to a lesser extent, factor IX, all with excellent safety records, are available in the world today. Most recombinant concentrates are made in the USA; a recombinant truncated factor VIII now is manufactured in Sweden and marketed through an international company, and a recombinant activated factor VII is made in, and marketed by, a company in Denmark. Sources of plasma-derived concentrate for export are the USA and several western European countries. American and European manufacturers have had access to sufficient plasma, including paid plasmapheresis plasma, from which to derive concentrates for domestic use as well as export for many years. A few countries have achieved self-sufficiency in volunteer donor plasma for limited periods of time. Cessation of self-sufficiency has come about not because of donor fatigue but because of consumer demand for recombinant concentrate or lack of consumer confidence in the safety of local plasma, as happened recently in the United Kingdom with the emergence of new variant Creutzfeld-Jakob disease (nvCJD).

Most concentrate made for export is manufactured primarily from plasma obtained from paid plasmapheresis donors, "source plasma". Some manufacturers also use plasma "recovered" from whole blood donated by unpaid volunteers. The major supplier of plasma from plasmapheresis is the USA, which has a huge and long-existing plasmapheresis industry. Additional source plasma is collected in Germany, Austria, Sweden and other European sources. Several European manufacturers depend on source plasma from the USA. The one commercial plasma fractionator in the United Kingdom has switched from the use of UK volunteer-donor plasma to the use of US paid-plasmapheresis plasma because of fear that nvCJD might be transmitted by plasma, a risk not yet substantiated. The capacity of some large fractionation plants is not now fully utilized. Some countries (Australia, Canada, Ireland, USA, UK, Ireland,

for example) are moving towards increased use of recombinant concentrates. In certain of these countries, domestic well-screened volunteer-donor plasma, a by-product of whole blood donations, may be discarded. Such countries are being urged to donate that plasma for conversion to plasma-derived concentrate for patients in less-wealthy nations. In other countries, recovered plasma still is being fractionated, sometimes on a small scale, for domestic use or, as in the USA, also for export.

Plasma collected by plasmapheresis is becoming ever safer. In the USA, donation sites have been moved from big cities to outlying suburbs and college towns with low endemic rates of hepatitis and HIV. This major, costly relocation scheme has, of itself, greatly reduced the rate of seropositivity for hepatitis and HIV of first-time donors. The sensitivity of serologic screening tests continues to improve. In the USA and most of Europe, plasma is now tested in small pools by genomic amplification techniques (GAT) such as PCR to detect hepatitis C virus and HIV earlier in the window period between beginning of infectivity of plasma and appearance of detectable antibodies. The European Union has mandated single-donor unit GAT, to improve sensitivity, and blood banks are striving to comply.

GAT is not useful for hepatitis B screening, in which antibodies appear as early as detectable virus. In the USA, the HIV epidemic has stabilized and GAT has not uncovered window-period cases of HIV contamination that were not already detected by p-24 antibody screening; GAT may replace p-24 antibody screening. In any country with an expanding HIV epidemic and a larger number of donors in the window period than in the USA, GAT may add information to that obtained by p-24 antibody testing. In such countries, p-24 antibody testing should be maintained if GAT cannot be implemented. GAT has detected window-period sero-negative cases of hepatitis C in plasma. More sensitive serologic tests for hepatitis C, under development, may obviate the need for GAT eventually.

As an additional precaution, source plasma in the USA is quarantined until the window period for appearance of antibodies to hepatitis and HIV has expired and the donor has returned and again tested negative. The safety of the

plasma going into manufacture has improved dramatically over the past decade.

Further measures are employed to prevent transmission of viral infections in plasma-derived concentrate. Purification and filtration steps remove virus by physical means. Viral-inactivation methods also are employed. Nowadays, two methods are often used. Treatment with solvent-detergent combinations remains popular, as does the use of heat, either while the concentrate is in solution ("pasteurization"), after lyophilization as a fine powder blown into steam vapor under pressure, or after lyophilization and bottling in the final container in a baking oven ("dry-heated"). Improvements in methods to stabilize of clotting factors to withstand heating at higher temperatures have improved the range of viruses that can be killed with dry heat. In several countries, recipients of concentrates are followed reasonably closely for seroconversion. No seroconversion for HIV has been reported in North America since 1987. Hepatitis B or C also are no longer transmitted by modern well-inactivated concentrates. A common virus, B-19 parvovirus, which is an occasional pathogen in susceptible recipients, is difficult to kill.

Technology that allowed measurement of viral kill started to develop about 20 years ago. Viral inactivation attempts began at the end of the 1970s in Germany, with pasteurization, to kill hepatitis viruses. Initially, the yield (the proportion of factor VIII in the starting plasma that survived the process) was very low, but the process was effective against hepatitis and, albeit unknown at the time, against HIV. Dry-heating began shortly afterwards in the USA, with the same goal of killing hepatitis viruses. Early dry-heat methods were ineffective against hepatitis and marginally effective against HIV, but the technology improved during the 1980s. These methods did not develop in the USA early enough to prevent the devastating HIV epidemic of the 1980s, but viral-inactivated plasma-derived concentrates of recent years have excellent safety records. New methods of viral inactivation are being explored. Some are suitable for cellular products.

Technologic developments opened options other than viral inactivation of plasma-derived concentrates. The human factor VIII gene was isolated and sequenced in the 1980s. It was transplanted into the nuclei of hamster cells, that is, re-combined with the nuclear material of those cells. In culture, these recombinant cells secrete human factor VIII, which then is harvested, purified, and concentrated. Recombinant factor VIII concentrate became available in the late 1980s in clinical trials. Animal and human plasma proteins were used in the culture medium, and human serum albumin was used for stabilization of the final product, giving rise to anxiety about the safety of those human derivatives. Later versions of recombinant factor VIII use human serum albumin only in the process of manufacture and use sugars for stabilization. A truncated factor VIII gene, coding for a shorter factor VIII molecule without the apparently unimportant B-domain, was created in the early 1990s. The altered clotting factor is expressed more abundantly in cell

culture than wild-type (normal) factor VIII. Recombinant B-domainless factor VIII concentrate is stabilized with sucrose. Recombinant factor IX always has been free of albumin stabilizer.

Because some consumers believed that human plasma was not to be trusted, recombinant concentrates became popular, indeed, were demanded, in some countries. In the USA, most hematologists use them as products of first choice for children, but most adults remain on plasma-derived concentrates. The popularity of recombinant concentrates exceeded the initial estimates of manufacturers and the products have been in short supply in the past year. Within five years, after construction of additional production facilities, an additional three to four billion units of recombinant factor VIII will be on the market, sufficient for an ample supply for developed countries. We shall soon have an abundance of factor VIII and IX concentrates, both plasma-derived and recombinant. We hope that some concentrate, most probably plasma-derived, can be made available at lower prices than the most common prices prevailing today to serve the needs of patients in less affluent countries.

The level of purification of factor VIII concentrates is no longer an issue of concern. Low- and intermediate-purity concentrates are used where appropriate. Highly purified factor VIII concentrates are in abundant supply in developed countries. For factor IX deficiency, prothrombin complex concentrate remains a mainstay of therapy. Single-factor concentrate of factor IX dominates the market in developed countries and is preferred everywhere for patients vulnerable to thrombosis, including those undergoing surgical operations. The size of the potential market is a major force driving development of special-purpose concentrates by commercial fractionators. The number of persons with inhibitors to factor VIII, which includes patients with hemophilia A and non-hemophilic patients with auto-antibodies, and the frequency of their hemorrhaging are sufficient to make the development of therapeutic products commercially viable. "By-passing" concentrates including activated prothrombin complex and recombinant activated factor VII, as well as porcine factor VIII, have been licensed for use in such patients.

Patients with von Willebrand disease continue to be served by factor VIII concentrates rich in von Willebrand factor, all of which concentrates are plasma-derived. Efforts have been made to produce a recombinant von Willebrand factor concentrate, but the market for such a product is limited to patients unresponsive to DDAVP or patients undergoing surgical operations in whom higher factor levels must be sustained than those provided by intermittent DDAVP stimulation.

Persons with less-common clotting factor deficiencies are served less well. The markets are small and the costs of licensure and marketing are large. Products for rare deficiencies have languished in the commercial sector with the exception of a factor VII concentrate manufactured for many years by Immuno Ag in Vienna and available on long-extended clinical trials. After the merger of Immuno with

Baxter, the latter has continued to supply the concentrate and now plans to proceed to licensure. Factor V is not concentrated in any product. No single-factor concentrate of factor X is being made; prothrombin complex is used for factor X deficiency. Concentrates of fibrinogen are available from a few sources. Fractionators which are or were part of a national blood system, such as Bio Products Laboratory in England and LFB (Laboratoire Français de Fractionnement et des Biotechnologies) in Lille, France, developed a greater array of unprofitable small-market concentrates (factor VII, factor XI, factor XIII) than did commercial fractionators. The cost of licensure prevents easy dissemination of such concentrates. We need to find ways to make such concentrates more easily available to the handful of patients who need them.

In some countries with very little money, the per-capita supply of concentrates is extremely low. A few of these countries have managed to produce some concentrate for domestic use, with simple viral inactivation methods such as solvent-detergent treatment (Rio de Janeiro, Brazil, unfortunately, now discontinued) or dry heat (Cuba, South Africa, Thailand).

Plasma and cryoprecipitate remain the mainstays of treatment in other countries and often are not as widely available as needed. Some of these poorer countries also have accelerating HIV epidemics. All transfusion recipients in such countries run risks of infection despite serologic testing, and those who must receive many transfusions face increased risks. In theory, if plasma is obtained by plasmapheresis of repeat donors and is quarantined until donors are re-tested after expiration of the window-period, single-donor cryoprecipitate and plasma might be safe. In practice, such programs are difficult to organize. Methods of viral inactivation suitable for sterilization of whole blood or whole plasma may be available in the near future and allow a modest level of self-sufficiency at an affordable cost in places with well-organized blood banks.

The heartening improvements in concentrates of the past two decades give us hope that the new millennium will bring a similarly rapid development of useful technology.

## References

- Kasper CK, Lusher JM, Transfusion Practices Committee: Recent evolution of clotting factor concentrates for hemophilia A and B. *Transfusion* 1993; 33:422-434
- Kasper CK: Plasma-derived versus recombinant factor VIII for the treatment of hemophilia A. *Vox Sang.* 1996; 70 (suppl 3): 17-20.
- Kasper CK, Costa e Silva M: Registry of clotting factor concentrates. *World Federation of Hemophilia, Facts and Figures* 1998, No.6
- Federici AB, Mannucci PM: Optimizing therapy with factor VIII/von Willebrand factor concentrates in von Willebrand disease. *Haemophilia* 1998; 4(suppl 3): 7-10
- Teitel, J. Safety of Coagulation Factor Concentrates. *Haemophilia* 1998, 4: 393-401.
- Miekka SI, Busby TF, Reid B, Pollock R, Ralston A, Drohan WN. New methods for inactivation of lipid-enveloped and non-enveloped viruses. *Haemophilia* 1998, 4: 402-408
- Bird A, Isarankura P, Almagro D, Gonzaga A, Srivastava A. Factor concentrates for haemophilia in the developing world. *Haemophilia* 1998, 4: 481-485
- World Federation of Hemophilia. Contract fractionation. *Facts and Figures* 1998, No.5.
- Evatt B. Creutzfeld-Jakob disease and hemophilia: Assessment of risk. *World Federation of Hemophilia, Treatment of Hemophilia*, 1999, No.15
- Funding rare and expensive treatments: The case of haemophilia. Report of a conference, King's Fund, October 23, 1997; *Haemophilia* 1998, 4 (suppl 1): 1-28.
- Kaufman RJ, Pipe SW. Can we improve on nature? "Super molecules" of factor VIII. *Haemophilia* 1998, 4: 370-379.