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TRANSMISSIBLE AGENTS AND THE SAFETY OF COAGULATION FACTOR CONCENTRATES

Revised Edition

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Transmissible Agents and the Safety of Coagulation Factor Concentrates

Jerome Teitel

Viral Transmission by Factor Concentrates

The transmission of pathogenic microorganisms continues to be a serious, but diminishing, risk associated with the transfusion of blood components. From the point of view of replacement therapy for hemophilia and related diseases, viruses have been the primary concern. The removal or inactivation of every virus particle in coagulation factor concentrates is a worthy goal in theory, but in practical terms it may be unnecessary and unattainable. In any event, absolute viral removal cannot be proven, as only a sample of the entire product is tested. From a practical perspective, the goal is to reduce pathogenic viral contamination to residual levels, where the virus is not infectious.

Separating viruses from blood components is difficult because of their size. Viral particles are smaller than other pathogens (disease-causing agents). In addition, some viruses are relatively resistant to inactivation techniques. Finally, new viruses periodically cross the species barrier undetected, and may enter the human blood supply. New disease-causing viruses would not be identified by monospecific screening tests, and they could be resistant to the viral reduction strategies in use.

Major Pathogenic Viruses Potentially Transmissible by Factor Concentrates

The major transmissible viruses that are present in plasma and cause serious and/or chronic disease are HIV, hepatitis B (HBV), and hepatitis C (HCV).

Other viral pathogens that can be transmitted by factor concentrates are of lesser concern. Parvovirus B19 is commonly transmitted by plasma-derived factor concentrates, but in adults at least, it generally causes mild disease that is without symptoms and is not considered clinically recognizable. Factor VIII concentrate has been implicated as the source of several limited outbreaks of infection with the hepatitis A virus (HAV). HAV also generally causes mild or subclinical disease, and it is not associated with a chronic hepatitis or with a persistent carrier state. In addition, effective vaccines are available to protect susceptible individuals. Both parvovirus B19 and HAV are small and lack a lipid envelope, characteristics that make them difficult to remove from plasma-derived products. They are also resistant to chemical inactivation by solvent-detergent reagents. They can, therefore, be considered "sentinel viruses," which could indicate the presence of other potentially dangerous viruses with similar physical properties.

Table 1:
Major Viruses Transmitted by Clotting Factor Concentrates

Virus	Size (Nm)	Genome	Envelope
HIV-1	90-100	RNA	Yes
HBV	40-45	DNA	Yes
HCV	40-60	RNA	Yes
HAV	25-30	RNA	No
B 19	18-20	DNA	No

Some transfusion-transmitted viruses, notably cytomegalovirus and HTLV-I, are not present in plasma, but only in the cellular matter of blood, and are therefore not relevant to coagulation factor concentrates.

Some characteristics of viruses transmitted by factor concentrates are shown in Table 1.

New, Emerging, or Potential Viral Threats to the Blood Supply

Hepatitis C infection accounts for most, but not all, cases of post-transfusion non-A, non-B hepatitis. This has naturally prompted a search for the causes of the remaining cases. The first credible candidate was hepatitis G virus (HGV). This virus has probably been transmitted via factor concentrates. It is likely that HGV is susceptible to the viral inactivation strategies that are effective for other enveloped viruses, although this remains to be established. Even if it is confirmed, the effectiveness of these procedures could be compromised if there are high initial viral levels in unscreened plasma pools. In any event, HGV's ability to cause disease as well as its tropism for liver cells remains uncertain. It is now considered unlikely that HGV is the cause of post-transfusion non-A, non-B hepatitis.

Other blood-borne viruses have been identified in recent years but, like HGV, have not been shown to cause disease in man. These include the transfusion-transmitted virus (TTV), a non-enveloped DNA virus, and the SEN-V virus.

West Nile virus has recently become established in North America, and has unequivocally been transmitted through the blood supply. As a lipid-enveloped virus it is sensitive to the viral inactivation procedures applied to clotting factor concentrates described below. Furthermore, nucleic acid testing for this virus is already being applied as a screening test to small donor pools.

Non-human viruses are a potential concern, but their ability to cause disease in humans is often unclear. For example, porcine factor VIII concentrate was recently found to be contaminated with porcine parvovirus (PPV). PPV is highly endemic in pig herds, but it is not known to be transmissible to humans. Laboratory and clinical data on recipients of porcine factor VIII have failed to demonstrate that it poses a health risk to humans. Attempts to exclude or inactivate PPV are complicated by its small size, and by the fact that porcine factor VIII is a fragile molecule that does not withstand the vigorous procedures needed to inactivate PPV. Therefore, screening of small plasma pools became the practical approach to prevent contamination of porcine factor VIII concentrate with PPV. Other animal viruses which have been recently become threats to human health include the Severe Acute Respiratory Syndrome (SARS)-related coronavirus and Simian Foamy Virus. Neither has been known to be transmitted by blood products, although the former agent has caused significant human disease.

General Principles for the Optimization of Viral Safety

A multifaceted approach that includes safety measures at every stage of production is needed to minimize the risk of viral transmission by coagulation factor replacement products. It includes screening donors, testing of donated blood, removal of viruses from therapeutic components, and inactivating viruses. Incorporating complementary safety measures further reduces the viral load entering the plasma pool and provides protection against manufacturing errors or oversights in any one of the steps. Responsibility for performing these steps lies with the manufacturers. Regulators are responsible for promulgating guidelines for viral safety (such as those of the Paul Ehrlich Institute or the Committee for Proprietary Medicinal Products) and for releasing the products for distribution. Treaters and consumers bear joint responsibility for ensuring that susceptible individuals are immunized against HBV and HAV, and that plasma-derived replacement products are used appropriately.

Limiting Exposure to Products with Potential Viral Contamination

The risk of viral transmission can be limited by using products that are not derived from human plasma in situations where it is safe and effective to do so. Unfortunately, most

hemophilia treaters can cite anecdotes in which treatment was based on misdiagnosed coagulation disorders, or concentrates were administered overly aggressively or for questionable indications, or outmoded and relatively unsafe replacement products were used. The use of clotting factor replacement therapy when pharmaceuticals, such as desmopressin or anti-fibrinolytic agents, may have been effective is also a common occurrence.

The use of recombinant clotting factor concentrates does not completely eliminate the risk of viral infection. Some recombinant products are formulated with albumin derived from human plasma. Albumin is produced using ethanol separation, and is pasteurized. The long experience with albumin as a volume expander attests to its safety, although it has not been subjected to the degree of scrutiny applied to clotting factor concentrates. More recently introduced recombinant factor VIII and factor IX concentrates are formulated with saccharide (sugar) stabilizers, removing the theoretical risk of albumin. However, even without albumin in the final formulation, these products could theoretically transmit human or non-human mammalian viruses. These could be introduced via the parent cell lines, or by human or animal proteins contained in the liquid media used to freeze or grow the cells.

Reducing the Initial Viral Load

The viral burden entering the plasma pool can be limited by careful donor selection and by testing individual donations for anti-viral antibody or for viral antigen (see Table 2). There is a common perception that the safest donor pool is one composed of altruistic volunteers. In fact, evidence shows that blood from selected repeat paid donors is less likely to have viral contamination. This illustrates the principle that the health of the donor population is the source of the viral problem, not the payment of donors for their blood. The selection process must be accompanied by strict criteria for the re-entry of deferred donors into the pool, and by a registry that effectively ensures that plasma from deferred donors is not inadvertently released.

Table 2:
Prevention of Virus Entry Into the Plasma Pool

Donor selection

- Self-deferral
- Deferral by centre

Screening of individual donor units

- Surrogate assay
- Antibody positivity
- Viral antigen

Screening of pool

- Viral nucleic acid

Donor re-testing

- Quarantine donor unit pending result

Specific viral screening tests are necessary in the production of plasma-derived concentrates, because subsequent viral removal or inactivation procedures can fail in the face of a heavily contaminated pool. However, given the limitations discussed below, it is evident that screening tests are not sufficient to insure optimal viral safety.

Traditionally, the most effective screening tools have been monospecific antibody or antigen detection tests for individual pre-selected virus targets. These tests should be periodically updated as technology improves. The priority of these tests is sensitivity, so that the ratio of true positive to false positive results should be low in selected (i.e., low prevalence) donor populations. In order to exclude false positives, all reactive sera should be subjected to a repeat screening test, followed by confirmatory testing. Nevertheless, the clinical significance of true antibody positivity can be problematic, as antibody is protective in some cases. This principle delayed the introduction of anti-HCV screening in the United States until 1991. There was real concern that removal of anti- HCV reactive units might compromise safety by eliminating protective antibody.

Viral antigen testing is available for HBV and HIV. Screening for HBV surface antigen (HBSAg) is extremely sensitive because large amounts of viral protein are synthesized early in the course of HBV infection. Still, HBV can be infectious at levels below the limit of detection by HBSAg tests. Furthermore, the sensitivity of the test may be reduced in the presence of antibody. In contrast to HBV, only small concentrations of HIV and HCV antigens circulate during early phases of infection, thus reducing their potential value as screening tests. In a large prospective American study, no positive HIV P24 antigen results were found in donor units that tested negative for HIV antibody. However, it is possible that in countries where there is a high incidence of newly acquired HIV infection, antigen testing may be useful in identifying some patients in the "window period," the early phase of infection before HIV can be detected by tests. With the introduction of HIV RNA screening tests, the value of P24 testing is doubtful, and is being phased out in some jurisdictions.

In the absence of clerical error or failure of quality control, false negative results are caused by sub-threshold viral contamination. This may occur during the window period early in the course of infection, or else in a late chronic carrier stage when the presence of the virus in the bloodstream or the host serological response has declined. Even though false negative results are rare, their significance may be greatly magnified, as a single virus positive unit can potentially contaminate a pool used to produce a large batch of factor concentrate.

One approach to reduce the risk of contamination during the window period is "donor re-testing," whereby frozen plasma is quarantined for sufficient time (e.g., three months) to allow retesting of donors who initially test negative. Another approach is the detection of viral genetic material by nucleic acid testing (NAT), which typically uses amplification techniques such as the polymerase chain reaction (PCR). Manufacturers of clotting factor concentrates have introduced NAT in recent years. The power of NAT is exemplified by the sensitivity of PCR testing for HBV, which is about six orders of magnitude (that is, about one million times) greater than that of antigen testing. However, NAT is applied not to individual units but to pools of varying sizes, so viral genome could be diluted below the detection limit of even this technique.

In principle, the potential utility of amplification techniques has been demonstrated in areas of high prevalence for HBV, where PCR testing has been able to identify positive (potentially infectious) individual donors who are HBSAg negative or antibody positive. With respect to HIV, PCR data have been reassuring to date. They suggest that donors with indeterminate antibody results, and donors who are at high risk but seronegative, are highly unlikely to be infectious. In practice, the benefit of adding NAT for HIV in factor concentrates will be difficult to demonstrate. Fewer than 30 cases of HIV transmission by antibody negative donor units were reported in the United States in the first decade after the introduction of testing. Even though this reported figure is likely to be an underestimate, the denominator was close to 150,000,000 units transfused. The current risk estimates for viral transmission by cellular blood products are under 1 per 100,000 for HBV and HCV, and under 1 per million for HIV. In clotting factor concentrates, this tiny residual risk is virtually eliminated by viral inactivation steps. Therefore, the incremental advantage of NAT is very small.

Nevertheless, NAT testing for HCV and HIV has been generally accepted as useful, whereas it would be expected to be of little benefit for HBV, in light of new HBSAg assays and anti-HBV core antibody testing. Parvovirus B19 is also a realistic target for NAT, given its relative resistance to viral inactivation methodologies.

The question of pool size often generates controversy, as it is dictated by considerations of commercial cost-effectiveness. Over time, most people with severe hemophilia will be exposed to factor concentrates produced from many different pools. On the one hand, the probability of viral contamination in a pool is directly proportional to the number of donors it includes. On the other hand, the concentration of virus introduced by a contaminated donation is inversely proportional to the pool size. Dilution to a lower viral titre might reduce the risk of transmission, and can also enhance the efficiency of viral inactivation.

Removal or Inactivation of Viruses

Techniques for viral removal or inactivation are not specific for individual agents, although their effectiveness may be partially or completely restricted to certain classes of viruses. Therefore, in contrast to the screening tests described above, it is not necessary to perform specific viral reduction steps in order to eliminate each known pathogen. In addition, viral removal or inactivation techniques can potentially reduce the risk of transmitting viruses whose presence in the donor pool may not have been known or suspected. However, the effectiveness of viral removal or inactivation has a limit. Some degree of viral kill must be compromised to avoid altering the clotting factor protein excessively. Therefore, these techniques complement donor selection and screening tests, but cannot replace them. Approaches for excluding and inactivating viruses in factor concentrates are summarized in Table 3.

Table 3:
Removal or Inactivation of Virus in the Plasma Pool

- Incidental removal during purification of protein of interest
- Specific viral removal by filtration
- Heat inactivation
  - 80-100°C x 0.5-72 hr
  - Pasteurization, 60°C x 10 hr
  - Heat under pressurized steam (60-80°C x 30-72 hr: OBSOLETE)
- Chemical inactivation
  - Solvent-detergent
  - Sodium thiocyanate
- Photochemical inactivation
  - Beta-propiolactone/UV
  - Ultraviolet-C

Physical separation of viruses from clotting factor concentrates occurs incidentally during their purification and formulation. Steps such as cryoprecipitation, chromatographic separation (including immunoaffinity chromatography), and lyophilization remove substantial amounts of virus.

There has been renewed interest in the application of filtration techniques to deliberately exclude viruses. In particular, the factor IX molecule is small enough to pass through ultrafiltration and nanofiltration membranes, which retain even HAV and parvovirus B19, the smallest disease-causing viruses, with little loss of product. These filters are now being used in the production of some factor IX concentrates. Although the larger size of the factor VIII molecule poses technical limitations, at least one Japanese plasma-derived factor VIII concentrate incorporates a nanofiltration step.

Specific viral inactivation procedures are applied to all plasma-derived coagulation factor concentrates. Some manufacturers are now applying them to recombinant concentrates as well, to further reduce the remote risk of viral transmission. Heat treatment is a widely used process, as viruses have varying sensitivity to heat. Unfortunately so do proteins, many of which (notably factor VIII) are readily altered in solution at 60C, the temperature used in pasteurization protocols. Unstable proteins are partially protected by the addition of chemical stabilizers, such as amino acids, citrate, or sugars, but losses of 10 to 15% of factor VIII activity are common. The effectiveness of heat as a treatment to inactivate viruses is a function of many factors, including time, temperature, physical state (dry or in solution), salt content, rate of temperature change, and nature and concentration of the stabilizers. In addition to pasteurization, heat is often applied to lyophilized concentrates at higher temperatures (80 to 100oC) for 0.5 to 72 hours. Heating such products to 60oC in hot vapour in an inert steam environment has an established safety record. With the exception of this process, temperatures below 80oC are relatively inefficient for inactivation of some model viruses in freeze-dried products.

Viruses that have lipid coats (including HIV, HBV, and HCV) can be efficiently inactivated by exposure to an organic solvent, usually tri-(n-butyl) phosphate (TNBP), in the presence of a detergent, either Tween 80, sodium cholate, or Triton X-100. As is the case for heat treatment, the effectiveness of solvent-detergent (S/D) protocols is time and temperature dependent. S/D causes rapid and complete inactivation of lipid-coated viruses, and the safety record of S/D-treated plasma fractionation products with

respect to these viruses is excellent. Pooled S/D-treated plasma recently became available as an alternative to single donor fresh frozen plasma for bleeding disorders for which viral-inactivated concentrates are not available. The SD-treated plasma product which was used in North America is no longer available, but a different product is still licensed in Europe. In any case, the relative advantages of S/D-treated plasma versus plasma produced from large pools should be considered on a case-by-case basis. This relates partly to the fact that the concentration of several coagulation-related proteins is different in S/D plasma than in normal plasma.

Recently, dual viral inactivation processes have been applied to factor concentrates, typically the addition of a terminal heat treatment step to S/D-treated products. This broadens the spectrum of viral inactivation to include non-enveloped viruses, while retaining the advantage of the potent activity of the S/D treatment. The experience to date with these dual-treated products has been good, with no evidence that the added manipulation has enhanced their likelihood to provoke an immune response. Triple viral reduction protocols, incorporating filtration with S/D and heat treatment, have also been investigated.

Other approaches to viral inactivation are based on chemical or photochemical methods. Sodium thiocyanate, a so-called chaotropic agent, has been successfully applied to factor IX concentrate, which is sufficiently stable to withstand the treatment. Photochemical treatment using methylene blue plus visible light has been used for viral inactivation in plasma for several years in Europe. Similar techniques, using ultraviolet irradiation of various wavelengths with or without added chemical sensitizing agents, have been applied to factor IX concentrates. Most of these procedures cannot be applied to unstable proteins such as factor VIII. Exposure to ultraviolet-C is an exception, which could become a useful supporting viral inactivation technique.

Costs of Viral Inactivation

The application of viral reduction strategies adds costs to clotting factor concentrates, both financial and otherwise. These procedures increase the complexity of the manufacturing process and reduce the yield of clotting factor, leading to increased monetary costs. The chemical agents that are added to concentrates in procedures such as S/D treatment are potentially toxic. It is important to ensure their removal from the final product. These procedures may also alter the clotting factor proteins in such a way as to render them less effective and/or more likely to provoke an immune response. This is not entirely a theoretical concern. Revision of a viral inactivation process applied to a plasma-derived factor VIII product in the Netherlands gave rise to a well-documented epidemic of factor VIII inhibitors, most of which were thankfully of low level and transient.

Interpretation of Viral Safety Data

Hemophilia treaters must interpret viral reduction data critically. It must not be assumed that log reductions achieved by individual fractionation and inactivation steps are necessarily additive, although data are often presented in a way that implies that this is the case. In practice, each step is evaluated individually for its ability to remove or inactivate virus "spiked" into the starting material. This experimental design is necessary, as there must be sufficient virus at each step for its viral reduction capacity to be measurable. However, as a result, interactions that may occur among the different methodologies are obscured. For example, different steps may not provide additional benefit if they preferentially inactivate the same subset of viral particles. In addition, some spiking studies use "model viruses," which may differ in subtle but important ways from the pathogens they are meant to mimic. Even when the authentic pathogens are used, cultured virus strains may behave differently from their wildtype counterparts. Finally, published viral reduction data are derived from small-scale experiments, and the results may not always be applicable to the larger production scale.

Surveillance for Transfusion-Transmitted Viral Disease

The recent impressive advances in the production of safe coagulation factor concentrates must not be allowed to create a sense of complacency in the hemophilia clinic. The ultimate proof of viral safety is not *in vitro* viral reduction data, but the demonstration that these concentrates do not transmit disease-causing viruses to susceptible individuals. Therefore, continued clinical and laboratory surveillance of the population receiving the concentrates is critical. This applies not only to the major known viruses, but also to the less threatening blood-borne viruses, and to those of doubtful significance. Careful surveillance and a high degree of suspicion will also allow the timely recognition of clinical events that may signal the entry of new viruses into the blood supply.

Non-viral Pathogens: Creutzfeldt-Jakob and Related Diseases

Transmissible spongiform encephalopathies (TSEs) are progressive and fatal neurological degenerative diseases that occur in many species. The human TSE is called Creutzfeldt-Jakob disease (CJD). The consensus is that TSEs are caused by infectious particles called prions, which are abnormal forms of normal proteins. Although the prion theory was promulgated relatively recently, the diseases are not new; some of them having been recognized for centuries. CJD itself was first described in 1920, before blood transfusion became commonplace.

Human prions have been transmitted by ritual consumption of human brain, injection of a brain hormone into muscle, transplantation of human corneas or dura mater (the membrane that covers the brain), and implantation into the brain of contaminated needles or electrodes. All these routes have one thing in common: they involve the inoculation or implantation of tissue from the brain or of structures appended to it. Brain and related tissues and organs are in fact the major sites in which prions can be found. However, lower concentrations do appear in whole blood and blood fractions.

Prions have been transmitted by blood in experimental animal models, giving rise to the theoretical possibility that CJD could be transmitted by blood. However, there is no epidemiologic or observational data suggesting that this has actually occurred. If CJD were a blood-borne illness, we would have expected to see cases of the disease in heavily transfused people, such as those with hemophilia or thalassemia. In fact, not a single case of CJD has been described in these groups. This is especially relevant because the estimated prevalence of asymptomatic "prionemia" (the presence of prions in the bloodstream) is up to 1 in 60,000, given the apparently prolonged incubation period of CJD. As the pools from which factor VIII and IX are fractionated may contain upwards of 60,000 donations, it is likely that most people with hemophilia who have received more than a few treatments with plasma-derived concentrates have been exposed to blood derivatives from affected donors. Many people in the general population have likely been exposed to such donors as well, through sources such as albumin in vaccines. Yet the incidence of sporadic CJD has not increased over the course of the twentieth century.

Prion diseases are generally difficult to transmit between species. One exception to this rule is BSE, the bovine TSE that is popularly known as mad cow disease. The BSE agent has entered the human food chain, and has caused a new illness in humans, referred to as "variant CJD" (vCJD). This disease has so far affected over 140 people in the United Kingdom, and small numbers have been reported elsewhere. No cases are thought to have been acquired in North America. Reported cases of vCJD have been distinguished from sporadic CJD by both clinical and pathological characteristics.

Variant CJD is only known to be transmitted by the oral route. The possibility of its transmission by blood transfusion is hypothetical. Even experimentally, most of the infectivity of blood is in the cellular components, not in plasma. However, the BSE agent that gave rise to vCJD may be more transmissible than other prions; it has crossed the species barrier via the food chain to infect not only humans, but a variety of other domestic and wild animals. Also, since vCJD is a new disease in humans, we have not observed it for long enough to be reassured that it does not

appear in transfusion recipients, as is the case for established diseases like sporadic CJD.

TSEs are always fatal, and there are currently no methods to screen asymptomatic donors, or to detect or inactivate the agents that cause them. Therefore, although the risk is still only hypothetical, it is important to maintain a high degree of suspicion and very close surveillance to protect the blood supply from possible transfusion-transmitted prion disease.