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Clinical perspectives of emerging pathogens in bleeding disorders

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As a result of immunological and nucleic-acid screening of plasma donations for transfusion-transmissible viruses, and the incorporation of viral reduction processes during plasma fractionation, coagulation-factor concentrates (CFC) are now judged safe in terms of many known infectious agents, including hepatitis B and C viruses, HIV, and human T-cell lymphotropic virus. However, emerging pathogens could pose future threats, particularly those with blood-borne stages that are resistant to viral-inactivation steps in the manufacturing process, such as non-lipid-coated viruses. As outlined in this Review, better understanding of infectious diseases allows challenges from newly described agents of potential concern in the future to be anticipated, but the processes of zoonotic transmission and genetic selection or modification ensure that plasma-derived products will continue to be subject to infectious concerns. Manufacturers of plasma-derived CFC have addressed the issue of emerging infectious agents by developing recombinant products that limit the need for human plasma during production. Such recombinant products have extended the safety profile of their predecessors by ensuring that all reagents used for cell culture, purification steps, and stabilisation and storage buffers are completely independent of human plasma.

Emerging pathogens could pose a substantial threat to recipients of therapeutic coagulation-factor concentrates (CFC), as shown by the devastating effect of HIV on patients who received these agents. The safety of CFC has improved greatly since their introduction, and the risk of infectious diseases caused by plasma-derived preparations is now very small. However, everyone concerned with haemophilia, including various scientific and medical advisory committees, agrees that vigilance must be maintained to ensure that new and unpredictable emerging blood-borne pathogens will not again compromise the safety of CFC in the future.^{1–3} The risk of transmission of infectious agents by a fresh blood component is very different from that for CFC because there might not be an effective pathogen inactivation or removal step in the preparation process, and the dose of infectious agent could be much higher than in CFC. The special danger of contamination of CFC with pathogens is that a single batch could be used to treat, and possibly infect, hundreds of patients, as happened with HIV. The pathogens discussed in this review are all potentially transmissible by fresh blood products. The susceptibility to individual pathogens in fresh blood products in a particular community will depend on the local epidemiology of the infectious agent. By contrast, CFC commonly cross continents from site of plasma collection and fractionation to use in recipients in a community with little experience of the agent. This review focuses on the potential for emerging risks of infectious agents in CFC because an essential public-health objective is to prevent epidemics of serious viral

infections in patients with haemophilia and others who depend exclusively on CFC for their well-being.

The review is a summary of an interdisciplinary forum of clinicians who treat haemophilia, infectious-disease specialists, and epidemiologists that was convened at Washington University School of Medicine in St Louis, MO, USA, in June, 2004, to discuss infectious-disease risk management in patients with haemophilia. The forum had the following objectives: to review emerging blood-borne pathogens and their potential effects on therapeutic CFC; to discuss clinical issues related to viral infections in patients with haemophilia; and to review the safety of CFC in terms of transfusion-transmissible infections.

Emergence of new human pathogens

The National Academy of Sciences defines an emerging infection as one that is newly identified, re-emerging, or drug resistant, with an incidence in people that has increased within the previous two decades or is threatening to increase in the near future.⁴ The first step of emergence, known as introduction, occurs when a previously unknown pathogen comes into contact with a new host population, or when an established pathogen changes its normal interaction pattern with its host population in some crucial way to increase in prevalence. In the second step, known as adaptation, the emerging agent becomes established and disseminates within the host population. The factors that contribute to this process are complex; they include microbial adaptation, advances in technology and industry, international travel and commerce, the breakdown of public-health measures, economic development and increased land use, changes in human demographics and behaviour, and war and famine.⁴

The introduction of a new pathogen into a previously unexposed population typically occurs by interspecies, or

Search strategy and selection criteria

The references were selected by the authors from their knowledge of published work.

zoonotic, transmission. For example, genetic analyses have suggested that HIV infection was transmitted to human beings from a common subspecies of chimpanzee (*Pan troglodytes troglodytes*).⁵ Another well-publicised example of zoonotic transmission is severe acute respiratory syndrome, caused by the transmission of a new coronavirus to people, initially in southern China and probably from small mammals, which are the natural hosts of the severe-acute-respiratory-syndrome coronavirus.⁶ After the virus first appeared in human beings, there was an explosive short epidemic. Since clinical symptoms preceded the peak of transmission of this virus, outbreaks were restricted by quarantine measures in China, Canada, and other countries. Many epidemiologists predict similar types of outbreaks in the future with emerging viruses.

A less widely known case of zoonotic transmission is of Nipah virus, a henipavirus that causes potentially lethal encephalitis. The natural host is the flying fox (fruit bat); cross-species transmission of the virus to animals occurred in southeast Asia where farmers inhabited areas that had been cleared of forests, inadvertently exposing their livestock to flying-fox habitats.^{7,8} In the late 1990s, there were several outbreaks of Nipah-virus infection in pigs, with subsequent transmission of the virus to human beings. In Malaysia, of 265 people infected during an outbreak of Nipah-virus infection, 105 died, a mortality rate of 40%.⁹ Two other outbreaks of Nipah-virus infection have since occurred in Bangladesh.^{7,8} The mortality rate among infected individuals approached 70% in the Bangladeshi outbreaks, and in contrast to the Malaysian outbreaks, the virus might have been transmitted from one person to another. Hendra virus, a type of henipavirus, causes disease in horses and was reported to be transmitted via exposure to secretions from an infected racehorse to its trainer. The transmission vector remains unknown but could be an arthropod.¹⁰

Any new viral pathogen poised to infect human beings must breach the natural barriers to zoonotic transmission that exist at the levels of cellular entry and viral expression. New emergent viruses might therefore have to acquire genetic mutations before active propagation within people is possible. Some RNA viruses have mutation rates as high as one nucleotide per 10 000 copied and therefore could be especially likely candidates for zoonotic transmission.¹¹ However, the evolution of a potentially large number of adaptive mutations that enable productive replication and efficient continuing transmission in a new host is rare, which might help to explain the low frequency of emerging agents. However, when an emerging pathogen does succeed in adapting to a new host species, the outcome can have a worldwide effect. New pathogens are also more likely to emerge in conjunction with opportunistic infections in highly immunosuppressed individuals, such as those with congenital

immune defects, cancer, or AIDS.¹² Immunosuppression, especially in xenotransplantation settings, might enable a poorly adapted virus to replicate and adapt to human beings and act as a bridge to subsequent infection of the healthy population.^{13,14}

These issues apply to infectious agents entering a new host population, but any pre-existing aetiological agent for which the incidence has increased within the previous two decades is also defined as an emerging pathogen. In many cases, an increase in incidence reflects a change in the interaction between host and vector, such as that caused by extraordinary changes in ecosystems.¹⁵ For example, Rift Valley fever was the ultimate cause of a cumulative 200 000 deaths after construction of the Aswan Dam on the River Nile in Egypt in the 1960s created conditions in which the arthropod vector flourished.¹⁶ Guanarito arenavirus emerged in Venezuela after forest clearing disrupted the local ecology.¹⁷ This viral pathogen can be transmitted from rats to human beings, in whom it causes haemorrhagic fever and can pose a threat to the blood supply. As more people congregate within mega-cities, especially those of less developed countries with less advanced hygiene systems, more emerging infectious agents that arise by disruption of ecosystems can be expected.

After introduction and adaptation, the final step in pathogen emergence is the dissemination of the emerging infectious agent within the host population. This step can occur with an explosive short outbreak, such as that observed for the Ebola virus or the coronavirus associated with severe acute respiratory syndrome. Alternatively, the agent can propagate and spread via a slower and more insidious mechanism, such as that observed with HIV.

Potential threats to blood and blood products

Many infectious agents transmissible by blood transfusion are characterised by a long-lasting and silent carrier state in which the pathogen circulates in the blood without causing noticeable symptoms.¹⁸ Blood

Panel: Viruses

CPV	Canine parvovirus
FPLV	Feline panleucopenia virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HTLV	Human T-cell lymphotropic virus
JEV	Japanese encephalitis virus
PCV	Porcine circovirus
SLE	St Louis encephalitis virus
TTMV	Torque-tenominivirus
TTV	Torque-tenovirus
WNV	West Nile virus
YFV	Yellow-fever virus

collected during this phase can be highly infective, even though the donor has no symptoms. Agents that meet these criteria include hepatitis B and C viruses (HBV and HCV), HIV, human T-cell lymphotropic viruses (HTLV) I and II, and West Nile virus (WNV).¹⁹ Because there is routine screening for these agents, blood and blood products are generally thought to be safe from these pathogens. However, many other viruses, for which donated blood is not routinely screened, are known to produce a viraemic phase in the infected individual. (Viruses for which we use an abbreviated name in this review are listed in the panel.)

Lipid-enveloped viruses

Flaviviruses, which are small, lipid-enveloped, positive-stranded RNA viruses, are a subfamily of arthropod-borne viruses (arboviruses) that cycle between insect and vertebrate hosts. At least 40 species are associated with human disease, including WNV, dengue virus, St Louis encephalitis virus (SLE), yellow-fever virus (YFV), and Japanese encephalitis virus (JEV).^{20,21} Human beings are the primary host for dengue virus and YFV only. Arboviruses have a vast global distribution and show intriguing emergence patterns based on ecological changes (figure 1).²² Because flaviviruses are widespread and persistent in insect and animal populations and are so far resistant to existing treatments, they pose a potential threat to the blood supply.

West Nile virus

This flavivirus is spread by mosquito vectors. The most important clinical phenotype of non-lethal infection is meningoencephalitis.²³ The virus first emerged during a 1999 outbreak in New York City, resulting in 62 confirmed cases and 22 deaths.^{24–26} During 2000 and 2001, similar numbers of human cases were noted in the eastern USA, and evidence of WNV found in birds, farm animals, and mosquito pools showed that the virus had spread over the whole eastern coast and had begun to spread westward. In 2003, the US Centers for Disease

Control and Prevention reported 9862 cases of WNV infection in 46 states and 264 associated deaths. The numbers were smaller in 2004, with 2470 reported cases in 41 states and 88 associated deaths. Infections have now been recorded in some parts of Canada and Mexico. Currently, 2% to 3% of people in certain areas of the USA are seropositive for WNV. The estimated risk, for example, of transmission of the virus through transfusion during the 1999 epidemic in New York was about 2·0 viraemic donations per 10 000 donors in the borough of Queens.²⁷ The risk varied greatly with time and location during 2002, reaching as high as 1·5 in 1000 donations.¹⁸ Of the 2·5 million blood donations screened for WNV from June to December, 2003, 0·05% were positive at first screen, and 0·02% were confirmed. Interventions to block transmission of WNV are hampered by the fact that 80% of cases of infection are subclinical or asymptomatic. Since WNV is readily inactivated by low pH, high temperature, and detergents, it is of less concern with plasma-protein-derived CFC, for which these treatments are typically used during manufacturing.

Dengue fever and dengue haemorrhagic fever

Dengue virus is the most common arthropod-transmitted virus in the world and is an important issue worldwide for the blood supply.²⁸ Each year, there are 25 million to 100 million cases of dengue fever and 250 000 cases of dengue haemorrhagic fever throughout the world.²⁹ Dengue haemorrhagic fever or dengue shock syndrome has a mortality rate of 10–40%, depending on the degree of access to high-quality tertiary-care facilities. Between 1980 and 1999, the number of cases of dengue virus in the Americas increased by 50 times (figure 2).³⁰ The primary mosquito vector for dengue virus, *Aedes aegypti*, was almost eradicated from the USA after the DDT spraying campaigns in the late 1950s but the population is now recovering.³¹ By comparison, in Brazil there is an epidemic of dengue fever and dengue haemorrhagic fever, with an estimated 500 000 to 1 000 000 cases per year. Other epidemics are occurring in Central America, southeast Asia, and parts of Africa and Oceania. Worldwide, an estimated 2·5 billion people are at risk. The infection has a viraemic phase that lasts 3–5 days, and most cases remain subclinical. Since no screening tests are in place, dengue virus might be expected to pose a future concern for the blood supply.

JEV

This virus is another important arbovirus threatening global blood supplies.^{32–34} It causes severe encephalitis, and the mortality among patients admitted to hospital is about 33%, with many survivors having long-term neurological sequelae. The heaviest concentrations of infections are in southeast Asia and the Indian subcontinent. 20 000–50 000 cases are reported every year in China alone.

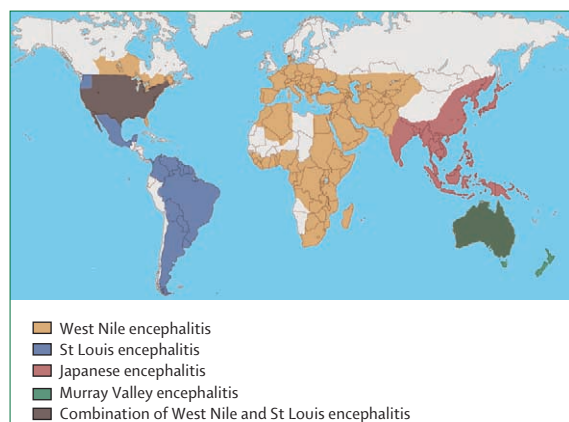


Figure 1: Approximate global distribution of medically important viruses in the Japanese encephalitis serogroup of flaviviruses²²

Non-lipid-enveloped viruses

Viruses without lipid envelopes tend to be less susceptible than lipid-enveloped viruses to inactivation methods because they are typically smaller in size and are likely to have greater resistance to heat, irradiation, and solvent detergent treatment. Emerging non-lipid-enveloped viruses could therefore pose the greatest threat to plasma-derived CFC, particularly in light of the recent outbreaks of infections with enteroviruses.³⁵

Enteroviruses

These small non-lipid-enveloped viruses form a separate genus within the picornavirus family. More than 70 serotypes have been isolated from people, including polioviruses, coxsackieviruses, enterocytopathic human orphan viruses, and enteroviruses 68–71. The normal site of replication for these viruses is the gastrointestinal tract; infection is subclinical or results in a mild disorder in many cases. However, some of these viruses can spread through the blood to other parts of the body, including the central nervous system. The resulting diseases can be severe, partly depending on the specific enterovirus serotype. Enteroviruses that undergo viraemic phases in their life cycle and are associated with substantial morbidity (eg, enteroviruses 70 and 71) could be particular threats to blood products.^{36–40} Enterovirus 70 was the causative agent of epidemics of acute haemorrhagic conjunctivitis in Africa, Asia, India, and Europe between 1969 and 1974. Enterovirus 71 seems to be highly pathogenic and neurovirulent and has been associated with epidemics (eg, in Taiwan, Japan, and Australia) of various acute diseases, including aseptic meningitis, encephalitis, paralytic poliomyelitis-like disease, hand-foot-and-mouth disease, herpangina, non-specific febrile illness, upper-respiratory-tract infection, enteritis, and viral exanthema.^{36–38} Enterovirus infections can have a transient viraemic phase that precedes symptoms. This feature, in combination with their resistance to inactivation, makes them a potential threat to the blood and CFC supplies. In a study in Scotland, 0.024% of blood donations showed evidence of enterovirus viraemia.⁴¹ This proportion predicts about 1000 enterovirus-contaminated transfusions per year in the UK. A wide range of viral loads (figure 3) and enterovirus serotypes were detected, including enterovirus 71, coxsackieviruses A2, A5, A10, A16, B2, B3, B4, and B5, and echoviruses 11, 13, 18, and 30. In the light of the numbers of potential infected donations, initiation of screening methods for enteroviruses might be prudent to exclude the possibility of blood-transfusion-associated transmission of potentially pathogenic variants such as enteroviruses 70 and 71.

Circoviruses

These viruses are the smallest known mammalian non-lipid-enveloped viruses (18–28 nm) and are distributed ubiquitously among human beings and other mammals.⁴²

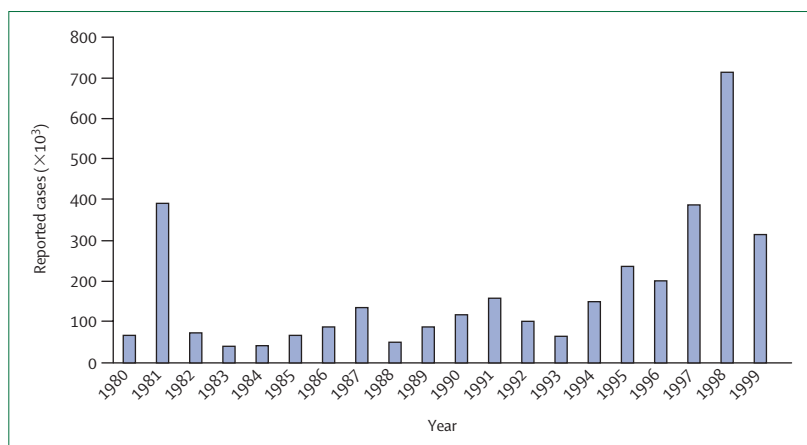


Figure 2: Reported cases of dengue fever in the Americas, 1980–99³⁰
Data for 1999 are provisional.

Human circoviruses include torque-tenovirus (TTV) and torque-tenominivirus (TTMV), two related viruses with highly divergent sequences. Each has a vast number of distinct genotypes. Infected people seem to be viraemic with a wide range of genotypes of both viruses, and viral loads are constantly high. Importantly for blood products, these extremely small and stable non-lipid-enveloped viruses cannot be removed easily by nanofiltration and as with other circoviruses important in veterinary medicine, such as porcine circovirus (PCV), they are likely to be highly resistant to heat and other viral inactivation protocols used in manufacture of blood products. There are no known human diseases associated with circoviruses, but some features suggest that caution is warranted. These viruses have been associated with clinical disease in other species; infection with PCV2 is the most commercially important disease in pigs today, leading to multisystemic wasting syndrome.⁴³ The virus is the cause of 15% of deaths in pigs, and it has already jumped species to cattle and sheep. Chicken anaemia virus, an avian circovirus, has been transmitted through contaminated manufactured livestock feed, a further

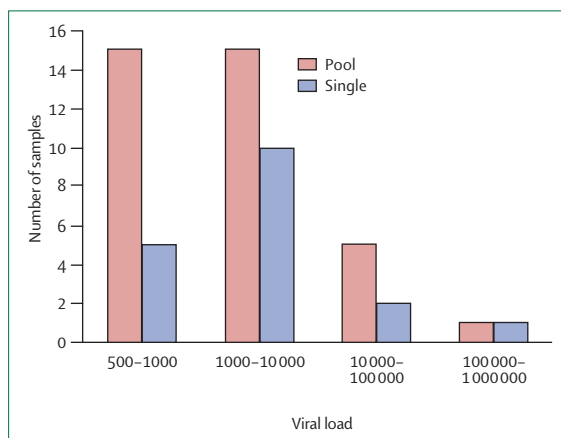


Figure 3: Reported viral loads of enterovirus types in Scottish donor plasma⁴¹

indication of the resistance to inactivation of these viruses. In human beings, transmission of circoviruses to neonates can be associated with upper-respiratory-tract infection.⁴⁴ Since these viruses might maintain their continuously viraemic but asymptomatic state in human beings through some immunosuppressive function, there is a theoretical concern that they might also suppress the immune response to other infectious agents.

Parvoviruses

Interspecies jumping is one of the primary routes by which new emerging pathogens enter the human population. The parvovirus feline panleucopenia virus (FPLV) might therefore constitute a future threat to people because of its ability to cross mammalian barriers. It is a small non-lipid-enveloped virus that typically infects cats, mink, raccoons, Arctic foxes, and raccoon dogs. In 1978, a new pathogenic variant emerged in dogs and became known as canine parvovirus (CPV),⁴⁵ which causes hyperthermia, vomiting, diarrhoea, leucopenia, dyspnoea, myocarditis, pneumonitis, and in some cases, virus-induced encephalitis.^{46,47} FPLV took about a decade to assume its new host range.^{48,49} One of the first changes that differentiated CPV from FPLV and other animal viruses was in the haemagglutinin molecule. Since then, strains of CPV have undergone a series of changes, presumably in response to evolutionary selection, which resulted in the global distribution of new variants.⁴⁸ New antigenic variants (CPV2 types, including 2a, followed by 2b then 2c) have been isolated from cats and dogs in Japan and Brazil, which suggests that interspecies transmission, and presumably genetic conversion, continues.⁵⁰⁻⁵² Host adaptation of CPV in canines suggests the possibility of similar cross-species transmission, followed by adaptation in human beings.⁵² This potential development could be accelerated by the close contact between dogs and people throughout the world. Because they are small, resistant, non-lipid-enveloped viruses, such emerging parvoviruses could pose a substantial future threat to blood and blood products.

Polyomaviruses

Polyomaviruses, a genus of the larger papovavirus family, are small non-lipid-enveloped viruses that might also pose a future risk to blood products. Two viruses, both of which were isolated in 1971, are of particular interest. JC virus was first isolated from the brain of a patient with Hodgkin's disease who subsequently developed progressive multifocal leucoencephalopathy. BK virus was first isolated from a susceptible immunosuppressed patient who had recently received a kidney transplant. The main site in the body found to harbour these viruses is the kidney. Lymphoid tissues, including bone marrow and spleen, have also been identified as sites of potential viral latency.⁵³⁻⁵⁵ Both viruses have also been found in brain tumours.⁵⁶

BK virus causes mild upper-respiratory-tract symptoms, occasional pyrexia, and transient cystitis in immuno-

competent individuals. In immunocompromised patients, the infection is predominantly associated with diseases of the urogenital tract, particularly haemorrhagic cystitis.⁵⁴ Nephropathy associated with BK virus is emerging as an important cause of renal dysfunction and loss of transplanted kidneys; the frequency of infection with BK virus in transplant recipients is 5%.^{57,58} Perhaps of greater concern, nucleic-acid sequences from the virus have been reported to persist in mononuclear cells of healthy blood donors. Thus, of 231 individuals tested in a study in two different centres, 29% were positive for BK-virus sequences.⁵⁴

Clinical infection with JC virus is generally found in association with long-term immunosuppression and other chronic diseases, including AIDS, lymphoproliferative diseases such as Hodgkin's disease and chronic lymphocytic leukaemia, sarcoidosis, tuberculosis, and systemic lupus erythematosus. JC virus causes chronic meningoencephalitis and is associated with progressive multifocal leucoencephalopathy, a fatal demyelinating disease of the central nervous system. Typically, the course of progressive multifocal leucoencephalopathy is gradual, with initial impairment of cognitive function, speech, vision, and movement. As it progresses, the pathology accelerates, and patients develop more severe disabilities, including dementia, paralysis, blindness, then coma, followed by death. The frequency of progressive multifocal leucoencephalopathy increased with the AIDS pandemic, but now, with effective antiretroviral therapy, HIV-infected patients rarely develop this disorder.^{51,52} Shedding of JC virus (and BK virus) in urine is significantly more common in HIV-infected patients than in non-HIV-infected individuals.⁵⁹ Progressive multifocal leucoencephalopathy is rare in children and young adults and is more common in people in their 50s and 60s; this feature suggests that reactivation of latent virus, and not primary infection, is the cause of viral disease.

Prions

Prions are self-replicating proteins implicated in transmissible spongiform encephalopathies in people and animals. The prion protein in its physiological form, PrP^c, is a normal membrane constituent. Its function is uncertain, although it might be involved in synaptic transmission or copper metabolism. The protein can undergo conformational change to a pathological form in which it is protease-resistant (PrP^{res}) and it might also have altered glycosylation. This conformational change increases the protein's ability to aggregate. This form is the agent that causes transmissible spongiform encephalopathies.

The most worrying manifestation of transmissible spongiform encephalopathies for people is variant Creutzfeldt-Jakob disease, which was first recognised in 1996.⁶⁰ The disease is caused by an infectious prion similar to that which causes bovine spongiform encephalopathy and is transmitted by consumption of

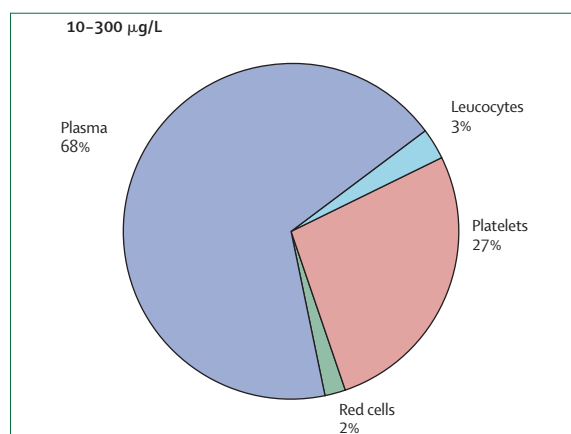


Figure 4: Quantification of PrP^{sc} protein in fractionated human blood⁶²

contaminated tissue from bovine sources. The prion that causes variant Creutzfeldt-Jakob disease is found in lymphoid tissue, in contrast to cases of the classic disease, and it therefore presents a potential risk of infection through blood and plasma-derived blood products.⁶¹

Several studies have shown that prions can be transmitted through blood. In one study, 68% of PrP^{sc} quantified in normal blood samples was in the plasma compartment, which highlights the potential for similar distributions of infectious prion. Leucocyte depletion alone might be insufficient to prevent transmission of PrP^{sc} (figure 4).⁶² Furthermore, sheep experimentally infected with the infectious prion associated with bovine spongiform encephalopathy or scrapie transmitted the encephalopathies to unexposed sheep at a rate of 10–20% after whole-blood donations. Finally, two human cases of probable transmission of the prion associated with variant Creutzfeldt-Jakob disease by blood from donors who subsequently developed the disorder have been reported. In the first case, the recipient received a transfusion in 1996, developed a neurological disorder in 2002, and died 13 months after the onset of symptoms. At autopsy, characteristic spongiform change was seen in the brain.⁶³ The second patient died 5 years after a blood transfusion from a donor who subsequently developed variant Creutzfeldt-Jakob disease. At autopsy, however, PrP^{sc} was found in the spleen and cervical lymph nodes, but there was no spongiform histological change or PrP^{sc} in the brain.⁶⁴

At present, variant Creutzfeldt-Jakob disease from infected blood donors poses mainly a theoretical risk to plasma-derived coagulation factors.⁶⁵ The risk could be less than that associated with fresh blood components because the pathogenic prion will be diluted in the starting plasma pool and because it is partly excluded by the differential protein fractionation process from the final CFC.⁶⁶ More highly purified CFC would probably carry a lower risk of prion transmission. However, without a reliable diagnostic test for prions, some concern about these agents will remain. In September, 2004,

serious concerns prompted the UK Departments of Health to issue letters to 6000 recipients of products manufactured from UK plasma, warning them that there was a possibility of transmission of variant Creutzfeldt-Jakob disease.

Public-health response

Regulatory bodies track adverse outcomes from the use of blood products, and public-health agencies track infectious disease, but surveillance for infectious agents in the blood supply is at an early stage and is not always done efficiently. Effective surveillance of blood products requires a coordinated effort by regulatory bodies, manufacturers, and treaters. Regulatory bodies must mandate effective postmarketing surveillance. Manufacturers must use their global reach to identify rare adverse events, and treaters must report adverse events as in the system established by UK Haemophilia Doctors' Organisation. Machine-readable labels such as those with bar codes, electronic health-record databases that track clinical outcomes, and consented tissue archives such as those of the US Centers for Disease Control and Prevention and the Blood Borne Pathogens Surveillance Project of the Association of Hemophilia Centre Directors of Canada are key tools for effective and efficient surveillance.⁶⁷ Table 1 lists some of these organisations.

Current clinical topics: viral disease in patients with haemophilia

Individuals with haemophilia and their physicians are particularly concerned about emerging blood-borne infectious diseases because the patients are continually exposed to blood and blood-derived products. The problems associated with HIV provide an example of the dangers faced by these people. HIV contamination of the

Region and organisation	Website
UK	
UK Haemophilia Alliance	http://www.haemophiliaalliance.org.uk
UK Haemophilia Society	http://www.haemophilia.org.uk
UK Haemophilia Centre Doctors' Organisation	http://www.ukhcdco.org
National Creutzfeldt-Jakob Disease Surveillance Unit	http://www.cjd.ed.ac.uk
Canada	
Association of Hemophilia Clinic Directors of Canada	http://www.ahcdc.ca/
Blood-Borne Pathogens Surveillance Project	http://www.ahcdc.ca/bbpsp
Canadian Hemophilia Society	http://www.hemophilia.ca/en/1.1.1.php
Blood-Borne Pathogen Surveillance Network	http://www.hc-sc.gc.ca/pphb-dgspsp/hcai-iamss/bpp-pts/sys_e.html
USA	
US National Hemophilia Foundation	http://www.hemophilia.org
Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases	http://www2.niaid.nih.gov/biodefense/research/rce.htm
Centers for Disease Control and Prevention	http://www.cdc.gov
International	
World Federation of Hemophilia	http://www.wfh.org

Table 1: Public-health resource organisations

blood supply was first recognised in 1983, although the first US case of HIV infection is now known to have occurred in 1975. By 1985, 74% of patients using factor VIII were seropositive.^{68,69} This degree of infection has had a profound effect on mortality among haemophilic patients. The median age at death among patients with haemophilia A decreased from 57 years before the HIV epidemic to 35 years in 1995, after the epidemic had grown to enormous proportions. By comparison, the median lifespan for HIV-negative haemophiliacs was 67 years in 1995.⁷⁰ Similarly, haemophilic patients previously have had high rates of infection with HBV and HCV. Virtually all patients treated with CFC before 1985 were exposed to HCV.⁷¹ In one study of HIV-negative adults, 5% had chronic HBV antigenaemia, 71% were seropositive for HBV, and 82% were seropositive for HCV.⁷² 25% of the deaths of HIV-infected patients were from liver disease in 1997 to 1999.⁷³

These observations highlight an important clinical issue for the treatment of haemophilic patients—that infections with several viruses complicate and confuse the clinical picture. For example, among HIV-infected individuals, subsequent HBV infection produces chronic disease in about 50% of cases, compared with 10% among HIV-negative patients.⁷⁴ HIV infection of a patient chronically infected with HBV, however, results in a higher degree of HBV replication. Nevertheless, these patients have milder liver inflammation and less liver fibrosis than HIV-negative individuals because the pathogenesis of chronic HBV infection is governed by immune-mediated mechanisms. Treatment of the immunodeficiency in these patients can accelerate liver disease, so antiretroviral therapy in patients infected with both HIV and HBV should include at least one agent that is active against HBV.

Similar types of interactions have been observed between HIV and HCV.⁷⁵ HIV infection increases the rate of progression to liver cirrhosis and decreases the response to interferon-based treatments.^{76,77} This last effect has complicated treatment recommendations for

these patients. If the immune system is well preserved (ie, the CD4-cell count is high), treatment of the HCV infection first might be prudent. If the patient has more advanced HIV disease, however, therapy for HIV should perhaps be started before that for HCV. The effect of infection with HCV on HIV progression remains uncertain; however, some studies suggest that patients infected with both viruses have accelerated progression of HIV disease and a blunted response to antiretroviral therapy.

Therapeutic CFC: protection from emerging infectious agents

Replacement therapy has formed the cornerstone of haemophilia treatment for longer than 100 years.⁷⁸ At first, whole blood was the source of exogenous coagulation factors. As treatment options evolved, whole blood was replaced with increasingly purified plasma product fractions, such as fresh-frozen plasma and cryoprecipitate, culminating in the high-purity, plasma-derived and recombinant products available now.

Since the available agents are highly effective, the choice of coagulation factor is driven primarily by product safety and cost concerns. Ultimately, cost-benefit analyses are needed to help physicians treating haemophilia to choose the best therapeutic option rationally. There have been no conclusive studies, but any pharmacoeconomic model will need to include some key principles. For example, because haemophilic patients need repeated infusions throughout their lives, the cumulative risk of transfusion-transmitted infectious diseases increases over their lifetimes, although currently the perceived overall threat is low. Furthermore, haemophilic patients are well informed about their disorder, and they might be expected to demand safer products when they hear about emerging infections. Given the complexities associated with choice of coagulation factor, clinicians and funders must understand the safety properties of the two main types of therapeutics, plasma-derived and recombinant coagulation factors.

Concentrate	Producing cell line	Factor VIII molecule	Addition of human or animal proteins to		Virus removal/inactivation method
			Cell culture/purification	Final formulation	
First generation					
RecombinateBioclone*	CHO	Full-length; coexpressed with VWF	Yes	Yes	Immunoaffinity, ion exchange
Kogenate/Helixate*	BHK	Full-length	Yes	Yes	Immunoaffinity, ion exchange, ultrafiltration
Helixate*					
Second generation					
ReFacto	CHO	B-domain deleted	Yes	No	Immunoaffinity, ion exchange, solvent detergent, nanofiltration
Kogenate FS/Helixate FS	BHK	Full-length	Yes	No	Immunoaffinity, ion exchange, solvent detergent, ultrafiltration
Helixate FS					
Third generation					
Advate	CHO	Full-length; coexpressed with VWF	No	No	Immunoaffinity, ion exchange, solvent detergent
ReFacto AF†	CHO	B-domain deleted	No	No	Immunoaffinity, ion exchange, solvent detergent, nanofiltration

WVF= von Willebrand factor. *No longer commercially available. †Not yet commercially available.

Table 2: Commercial recombinant factor VIII products

Plasma-derived coagulation factors

Human plasma-derived CFC were one of the original products used in the treatment of haemophilia. Each year, tens of thousands of patients receive plasma-derived products.⁷⁹ However, for longer than three decades, plasma-derived CFC have been known to transmit infectious pathogens.⁸⁰ Human plasma donations are therefore routinely screened for known infectious agents by antigen, antibody, and nucleic-acid testing procedures. Compared with whole blood or plasma, CFC have the additional safety advantage of undergoing plasma fractionation and are subject to procedures to reduce numbers of viruses during manufacture, including chromatographic fractionation, nanofiltration, solvent detergent treatment, and heat inactivation. Thus, plasma-derived coagulation factors now have a very low risk of transfusion-mediated infection with HBV, HCV, HIV, and HTLV I and II. We emphasise, however, that none of the current viral reduction steps in the manufacture of concentrates eliminate the risk of transmission of non-lipid-coated viruses. Plasma-derived CFC have been widely available for 30 years. They are effective at preventing and stopping haemorrhage in most patients with haemophilia and have therefore revolutionised the lives of patients. Many are able to treat themselves at home. With regular injections in childhood, most bleeds can be prevented, allowing children to grow up with normal joints and not, as was previously commonplace, crippled with arthritis.

Recombinant coagulation factors

The threat from unknown emerging infectious agents has encouraged manufacturers to remove dependency on pooled blood plasma entirely from the manufacturing process. They have therefore developed methods for expressing human genes for coagulation factors within immortalised tissue-culture cells. Subsequent immunoaffinity and column-chromatographic purification and viral inactivation and removal steps result in highly purified therapeutic preparations that are fully active. These processes decrease the likelihood of contamination by emerging infectious agents. There is a possibility, however, that the cell line could be infected with, and propagate, a pathological prion.⁸¹ The first recombinant factor VIII concentrate was marketed in 1992 for the treatment of haemophilia A. Other products followed (table 2).

The first-generation recombinant concentrates were manufactured from cultures that contained animal and human proteins. Human albumin was also added as an excipient to the final preparation. In the second generation, protein stabilisers such as albumin were replaced with sucrose to eliminate the potential of pathogen transmission. Recombinant factor VIIa is also a second-generation product used to treat haemophiliacs who have antibodies to factor VIII and do not respond to factor VIII concentrates.

In third-generation concentrates, the factor-VIII-transfected cell line is grown in medium without addition of any animal or human proteins.² This approach has greatly reduced the risk that could be posed by future emerging pathogens. The only licensed recombinant factor IX concentrate, BeneFIX, is also a third-generation concentrate. To improve safety in the manufacture of recombinant concentrates, all manufacturing processes now include a virus-inactivation process, such as solvent detergent. In addition, the monoclonal antibodies used to purify factor VIII from cell cultures are made from hybridoma cell lines grown in the absence of added mammalian protein. Although recombinant CFC are judged to be at lower risk of transmitting infectious agents, concern has been expressed that they might be associated with a higher incidence of alloantibody development in response to the transfused factor VIII.⁸² Future gene-transfer technologies might allow the preparation of human clotting-factor proteins by novel and high-yielding processes that would enable the native protein to be expressed.

Conflict of interest statement

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References

- 1 Association of Hemophilia Clinic Directors of Canada. Hemophilia and von Willebrand's disease: 1, diagnosis, comprehensive care and assessment. *Can Med Assoc J* 1995; **153**: 19–25.
- 2 United Kingdom Haemophilia Centre Doctors' Organisation. Guidelines on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders. *Haemophilia* 2003; **9**: 1–23.
- 3 National Hemophilia Foundation. MASAC recommendations concerning the treatment of hemophilia and other bleeding disorders. 2003: Document 151.
- 4 Smolinski M, Hamburg M, Lederburg J. Microbial threats to health: emergence, detection, and response. Washington, DC: National Academies Press, 2003.
- 5 Vandamme AM, Liu HF, Van Brussel M, De Meurichy W, Desmyter J, Goubau P. The presence of a divergent T-lymphotropic virus in a wild-caught pygmy chimpanzee (*Pan paniscus*) supports an African origin for the human T-lymphotropic/simian T-lymphotropic group of viruses. *J Gen Virol* 1996; **77**: 1089–99.
- 6 Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 2003; **302**: 276–78.
- 7 Butler D. Fatal fruit bat virus sparks epidemics in southern Asia. *Nature* 2004; **429**: 7.
- 8 Enserink M. Emerging infectious diseases. Nipah virus (or a cousin) strikes again. *Science* 2004; **303**: 1121.
- 9 Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science* 2000; **288**: 1432–35.
- 10 Westbury HA. Hendra virus disease in horses. *Rev Sci Tech* 2000; **19**: 151–59.
- 11 Monk RJ, Malik FG, Stokesberry D, Evans LH. Direct determination of the point mutation rate of a murine retrovirus. *J Virol* 1992; **66**: 3683–89.

- 12 Weiss RA, McMichael AJ. Social and environmental risk factors in the emergence of infectious diseases. *Nat Med* 2004; **10** (12 suppl): S70–76.
- 13 Patience C, Takeuchi Y, Weiss RA. Zoonosis in xenotransplantation. *Curr Opin Immunol* 1998; **10**: 539–42.
- 14 Takeuchi Y, Patience C, Magre S, et al. Host range and interference studies of three classes of pig endogenous retrovirus. *J Virol* 1998; **72**: 9986–91.
- 15 United Nations Environment Programme. Geo Yearbook 2004/5: Available at: http://www.grida.no/geo/pdfs/geo_yearbook_2004_eng.pdf (accessed Sept 7, 2005).
- 16 Meegan JM, Hoogstraal H, Moussa MI. An epizootic of Rift Valley fever in Egypt in 1977. *Vet Rec* 1979; **105**: 124–25.
- 17 Salas R, de Manzione N, Tesh RB, et al. Venezuelan haemorrhagic fever. *Lancet* 1991; **338**: 1033–36.
- 18 Dodd RY, Leiby DA. Emerging infectious threats to the blood supply. *Annu Rev Med* 2004; **55**: 191–207.
- 19 Busch MP, Kleinman SH, Nemo GJ. Current and emerging infectious risks of blood transfusions. *JAMA* 2003; **289**: 959–62.
- 20 Solomon T, Winter PM. Neurovirulence and host factors in flavivirus encephalitis: evidence from clinical epidemiology. *Arch Virol Suppl* 2004; **18**: 161–70.
- 21 Scaramozzino N, Crance JM, Jouan A, DeBriel DA, Stoll F, Garin D. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. *J Clin Microbiol* 2001; **39**: 1922–27.
- 22 Solomon T. Flavivirus encephalitis. *N Engl J Med* 2004; **351**: 370–78.
- 23 Solomon T, Ooi MH, Beasley DW, Mallewa M. West Nile encephalitis. *BMJ* 2003; **326**: 865–69.
- 24 Komar N. West Nile virus: epidemiology and ecology in North America. *Adv Virus Res* 2003; **61**: 185–234.
- 25 Pomper GJ, Wu Y, Snyder EL. Risks of transfusion-transmitted infections: 2003. *Curr Opin Hematol* 2003; **10**: 412–18.
- 26 Huhn GD, Sejar JJ, Montgomery SP, Dworkin MS. West Nile virus in the United States: an update on an emerging infectious disease. *Am Fam Physician* 2003; **68**: 653–60.
- 27 Biggerstaff BJ, Petersen LR. Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. *Transfusion* 2002; **42**: 1019–26.
- 28 Mairuhu AT, Wagenaar J, Brandjes DP, van Gorp EC. Dengue: an arthropod-borne disease of global importance. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 425–33.
- 29 World Health Organization. Strengthening implementation of the global strategy for dengue fever/dengue haemorrhagic fever prevention and control. Available at: <http://www.who.int/csr/resources/publications/dengue/whocdsdnic20001.pdf> (accessed Sept 7, 2005).
- 30 Centers for Disease Control and Prevention. Division of Vector-Borne Infectious Diseases. Dengue fever. <http://www.cdc.gov/ncidod/dvbid/dengue/index.htm> (accessed Jan 12, 2006).
- 31 Reiter P, Lathrop S, Bunning M, et al. Texas lifestyle limits transmission of dengue virus. *Emerg Infect Dis* 2003; **9**: 86–89.
- 32 Solomon T, Vaughn DW. Pathogenesis and clinical features of Japanese encephalitis and West Nile virus infections. *Curr Top Microbiol Immunol* 2002; **267**: 171–94.
- 33 Solomon T, Mallewa M. Dengue and other emerging flaviviruses. *J Infect* 2001; **42**: 104–15.
- 34 Tiroumourogane SV, Raghava P, Srinivasan S. Japanese viral encephalitis. *Postgrad Med J* 2002; **78**: 205–15.
- 35 Chan YF, AbuBaker S. Recombinant human enterovirus 71 in hand, foot and mouth disease patients. *Emerg Infect Dis* 2004; **10**: 1468–70.
- 36 Chang LY, Lin TY, Huang YC, et al. Comparison of enterovirus 71 and coxsackie-virus A16 clinical illnesses during the Taiwan enterovirus epidemic, 1998. *Pediatr Infect Dis J* 1999; **18**: 1092–96.
- 37 Chang LY, Tsao KC, Hsia SH, et al. Transmission and clinical features of enterovirus 71 infections in household contacts in Taiwan. *JAMA* 2004; **291**: 222–27.
- 38 Komatsu H, Shimizu Y, Takeuchi Y, Ishiko H, Takada H. Outbreak of severe neurologic involvement associated with enterovirus 71 infection. *Pediatr Neurol* 1999; **20**: 17–23.
- 39 Huang CC, Liu CC, Chang YC, Chen CY, Wang ST, Yeh TF. Neurologic complications in children with enterovirus 71 infection. *N Engl J Med* 1999; **341**: 936–42.
- 40 Li CC, Yang MY, Chen RF, et al. Clinical manifestations and laboratory assessment in an enterovirus 71 outbreak in southern Taiwan. *Scand J Infect Dis* 2002; **34**: 104–09.
- 41 Welch J, Maclaran K, Jordan T, Simmonds P. Frequency, viral loads, and serotype identification of enterovirus infections in Scottish blood donors. *Transfusion* 2003; **43**: 1060–66.
- 42 Hino S. TTV, a new human virus with single stranded circular DNA genome. *Rev Med Virol* 2002; **12**: 151–58.
- 43 Fenaux M, Halbur PG, Gill M, Toth TE, Meng XJ. Genetic characterization of type 2 porcine circovirus (PCV-2) from pigs with postweaning multisystemic wasting syndrome in different geographic regions of North America and development of a differential PCR-restriction fragment length polymorphism assay to detect and differentiate between infections with PCV-1 and PCV-2. *J Clin Microbiol* 2000; **38**: 2494–503.
- 44 Maggi F, Pifferi M, Fornai C, et al. TT virus in the nasal secretions of children with acute respiratory diseases: relations to viremia and disease severity. *J Virol* 2003; **77**: 2418–25.
- 45 Dobson A, Foufopoulos J. Emerging infectious pathogens of wildlife. *Philos Trans R Soc Lond B Biol Sci* 2001; **356**: 1001–12.
- 46 Bastianello SS. Canine parvovirus myocarditis: clinical signs and pathological lesions encountered in natural cases. *J S Afr Vet Assoc* 1981; **52**: 105–08.
- 47 Baatz G. [Ten years of clinical experiences with canine parvovirus infection CPV-2 infection]. *Tierarztl Praxis* 1992; **20**: 69–78.
- 48 Parrish CR. Host range relationships and the evolution of canine parvovirus. *Vet Microbiol* 1999; **69**: 29–40.
- 49 Truyen U, Parrish CR. Canine and feline host ranges of canine parvovirus and feline panleukopenia virus: distinct host cell tropisms of each virus in vitro and in vivo. *J Virol* 1992; **66**: 5399–408.
- 50 Pereira CA, Monezi TA, Mehnert DU, D'Angelo M, Durigon EL. Molecular characterization of canine parvovirus in Brazil by polymerase chain reaction assay. *Vet Microbiol* 2000; **75**: 127–33.
- 51 Mochizuki M, Harasawa R, Nakatani H. Antigenic and genomic variabilities among recently prevalent parvoviruses of canine and feline origin in Japan. *Vet Microbiol* 1993; **38**: 1–10.
- 52 Ikeda Y, Nakamura K, Miyazawa T, Takahashi E, Mochizuki M. Feline host range of canine parvovirus: recent emergence of new antigenic types in cats. *Emerg Infect Dis* 2002; **8**: 341–46.
- 53 Seth P, Diaz F, Major EO. Advances in the biology of JC virus and induction of progressive multifocal leukoencephalopathy. *J Neurovirol* 2003; **9**: 236–46.
- 54 Dolei A, Pietropaolo V, Gomes E, et al. Polyomavirus persistence in lymphocytes: prevalence in lymphocytes from blood donors and healthy personnel of a blood transfusion centre. *J Gen Virol* 2000; **81**: 1967–73.
- 55 Berger JR, Major EO. Progressive multifocal leukoencephalopathy. *Semin Neurol* 1999; **19**: 193–200.
- 56 Croul S, Otte J, Khalili K. Brain tumors and polyomaviruses. *J Neurovirol* 2003; **9**: 173–82.
- 57 Mylonakis E, Goes N, Rubin RH, Cosimi AB, Colvin RB, Fishman JA. BK virus in solid organ transplant recipients: an emerging syndrome. *Transplantation* 2001; **72**: 1587–92.
- 58 Vats A, Shapiro R, Singh Randhawa P, et al. Quantitative viral load monitoring and cidofovir therapy for the management of BK virus-associated nephropathy in children and adults. *Transplantation* 2003; **75**: 105–12.
- 59 Behzad-Behbahani A, Klapper PE, Vallery PJ, Cleator GM, Khoo SH. Detection of BK virus and JC virus DNA in urine samples from immunocompromised (HIV-infected) and immunocompetent (HIV-non-infected) patients using polymerase chain reaction and microplate hybridisation. *J Clin Virol* 2004; **29**: 224–29.
- 60 Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996; **347**: 921–25.
- 61 Hill AF, Zeidler M, Ironside J, Collinge J. Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet* 1997; **349**: 99–100.

- 62 MacGregor I, Drummond O. Immunoassay of human plasma cellular prion protein. *Transfusion* 2001; **41**: 1453–54.
- 63 Llewelyn CA, Hewitt PE, Knight RS, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 2004; **363**: 417–21.
- 64 Peden A, Head M, Ritchie D, Bell J, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterogenous patient. *Lancet* 2004; **364**: 527–29.
- 65 Ludlam CA, Turner ML. Managing the risk of transmission of variant Creutzfeldt Jakob disease by blood products. *Br J Haematol* 2006; **132**: 13–24.
- 66 Lee DC, Stenland CJ, Miller JL, et al. A direct relationship between the partitioning of the pathogenic prion protein and transmissible spongiform encephalopathy infectivity during the purification of plasma proteins. *Transfusion* 2001; **41**: 449–55.
- 67 Ritchie B. Tissue archives to track blood borne pathogens in people receiving blood products. *Transfus Apheresis Sci* 2003; **29**: 269–74.
- 68 Poon MC, Landay A, Prasthofer E, Stagno S. Acquired immunodeficiency syndrome with *Pneumocystis carinii* pneumonia and *Mycobacterium avium-intracellulare* infection in a previously healthy patient with classic hemophilia: clinical, immunologic, and virologic findings. *Ann Intern Med* 1983; **98**: 287–90.
- 69 Jason J, McDougal JS, Holman RC, et al. Human T-lymphotropic retrovirus type III/lymphadenopathy-associated virus antibody: association with hemophiliacs' immune status and blood component usage. *JAMA* 1985; **253**: 3409–15.
- 70 Soucie JM, Nuss R, Evatt B, et al. Mortality among males with hemophilia: relations with source of medical care. *Blood* 2000; **96**: 437–42.
- 71 Makris M, Baglin T, Dusheiko G, et al. Guidelines on the diagnosis, management and prevention of hepatitis in haemophilia. *Haemophilia* 2001; **7**: 339–45.
- 72 Diamondstone LS, Aledort LM, Goedert JJ. Factors predictive of death among HIV-uninfected persons with haemophilia and other congenital coagulation disorders. *Haemophilia* 2002; **8**: 660–67.
- 73 Darby SC, Kan SW, Spooner RJ, et al. The impact of HIV on mortality rates in the complete UK haemophilia population. *AIDS* 2004; **18**: 525–33.
- 74 Thio CL. Management of chronic hepatitis B in the HIV-infected patient. *AIDS Read* 2004; **14**: 122–29, 133, 136–37.
- 75 Rockstroh JK, Spengler U. HIV and hepatitis C virus co-infection. *Lancet Infect Dis* 2004; **4**: 437–44.
- 76 Chung R, Andersen J, Volberding P, et al. Peginterferon alfa-2a plus ribavirin versus interferon alfa 2a plus ribavirin for chronic hepatitis C in HIV-coinfected persons. *N Engl J Med* 2004; **351**: 451–59.
- 77 Torriani F, Rodriguez-Torres M, Rockstroh JK, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *N Engl J Med* 2004; **351**: 438–50.
- 78 Hilgartner MW. The need for recombinant factor VIII: historical background and rationale. *Semin Hematol* 1991; **28** (suppl 1): 6–9.
- 79 Chamberland ME. Emerging infectious agents: do they pose a risk to the safety of transfused blood and blood products? *Clin Infect Dis* 2002; **34**: 797–805.
- 80 Hoots WK. History of plasma-product safety. *Transfus Med Rev* 2001; **15** (suppl 1): 3–10.
- 81 Vorberg I, Raines A, Story B, Priola SA. Susceptibility of common fibroblast cell lines to transmissible spongiform encephalopathy agents. *J Infect Dis* 2004; **189**: 431–39.
- 82 Rothschild C, Goudemand J, Demiguel V, Calvez T. Effect of type of treatment (recombinant vs. plasmatic) on FVIII inhibitor incidence according to known risk cofactors in previously untreated severe haemophilia A patients (Pups). *J Thromb Haemost* 2003; **1** (suppl): abstr OC215.