Emerging Viral Infections - A Potential Threat for Blood Supply in the 21st Century

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Abstract

During the last 25 years the safety of blood products has improved dramatically with regard to infectious risk, notably to the threat represented by retroviruses (HIV and human T-cell lymphotropic virus) and hepatitis B and C viruses. However, both residual and emergent viral infections are still responsible for contaminations in recipients of blood products. Along with other viruses (human herpesvirus-8, human parvovirus B19, hepatitis A and E viruses, etc.), special attention has recently been paid to emerging arboviruses, such as West Nile virus in North America, and Dengue and Chikungunya viruses in Europe. Another blood-linked risk, notably in the United Kingdom and France, is the prion agent responsible for the variant form of the Creutzfeldt-Jakob disease. Hemophilia care has been the model for improvements in the safety and availability of safe blood components free of infectious agents. In this regard, several measures aimed to halt transmission of viruses have been implemented in blood banks, including the exclusion of at-risk donors, specific sensitive diagnostic tests, leukocyte reduction of labile blood products, and the physical or chemical treatments aiming at nonspecific inactivation of infectious agents potentially present in blood without impairing significantly its physiological properties. **(AIDS Rev. 2012;14:279-89)**

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Introduction

Transfusion-transmitted infections remain a major subject of interest for blood safety. Most emerging infectious diseases are caused by zoonotic pathogens. The number and proportion that originate in wild animals in particular has increased substantially in the past few decades, even after accounting for increased reports of new emerging infectious diseases[1-4]. Trends in globalization, including expansion in international travel and trade, have also extended the reach and increased the pace at which infectious diseases spread[5], prompting the need for more rapid outbreak detection and reporting along with improved transparency to minimize the burden on global health and the economy.

In this context, a spectrum of blood viral infectious agents is transmitted through transfusion of infected blood donated by apparently healthy and asymptomatic blood donors[7]. The diversity of blood-borne infectious agents includes hepatitis B virus (HBV), hepatitis C virus (HCV), HIV-1/2, human T-cell lymphotropic virus (HTLV-1/2), cytomegalovirus (CMV), human parvovirus B19, West Nile virus (WNV), Dengue virus (DENV), human herpes virus-8 (HHV-8), and variant of Creutzfeldt-Jakob disease (vCJD)[6,7].

In addition, little is known about the risk of transmitted-transfusion for new emerging viral infections. For example, an outbreak of Hantavirus infection in the

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Yosemite National Park (California, USA) occurred last summer. A total of nine cases were confirmed and three of them were fatal. More than 230,000 overnight visitors, national and international, were advised about the symptoms of hantavirus pulmonary syndrome, a rare but serious illness caused by hantavirus, resulting in an unprecedented number of medical consultations around the world[8]. Other viruses currently spreading and for which no specific measures have been taken so far to protect blood safety are the following: Chikungunya, that now causes outbreaks outside African countries, hepatitis E virus (HEV), new variants of HIV-1, such as group P, and HTLV, such as 3 and 4, or simian foamy viruses[8,9]. Moreover, many other pathogens that are not viruses, such as *Babesia* spp, *Tripanosoma cruzi* (Chagas disease) or *Plasmodium* sp (malaria), have the potential to be transmitted by transfusion since they have an asymptomatic blood-borne phase and may survive in blood components during processing and storage[6,7].

Hemophilia care has been one of the main drivers in the evolution through safer blood donations during the past 50 years. Hemophilia A and B are hereditary X-chromosomal recessive disorders caused by deficiency or absence of coagulation factors VIII and IX, respectively. The causative mechanism of hemophilia was recognized in the 1950s, although early concentrates were not available and sufficiently refined to enable efficient self-administered treatment at home until the 1970s. The advances, allowing easier administration, also resulted in transmission of viral diseases, initially including hepatitis and thereafter HIV, leading to a tremendous mortality in the hemophilia population during the period 1985-1995. The availability of screening assays for identifying viral infections, followed by viral inactivation plasma procedures, and finally the advent of recombinant concentrates has greatly improved the safety and availability of hemophilia treatment[10].

In this review, we will focus in new emerging viral infections and their potential risk of transmission by blood transfusion as well as the interventions and alternatives for safer blood transfusions.

Virus classification and blood-borne viruses

The three domains of cellular life are bacteria, archaea, and eukarya. Beyond are viruses, which are obligated parasites, constituted by a nucleic acid encapsidated by proteins making the envelope. The whole virosphere can be classified based on three

principles: (i) nature of the genome (single- or double-stranded DNA or RNA) proposed by Baltimore (1971); (ii) taxonomy by genetic phylogeny (order, family, genus and species) following genomic relatedness proposed by the International Committee on Taxonomy of Viruses (2005); and (iii) viral capsid structure (icosahedral, helical or their combination) (2012)[11]. Most of the emergent viral infections are caused by RNA and/or non-enveloped agents. Both characteristics facilitate a rapid adaptation to the host that then becomes a potential source for pandemic infections.

In addition, all viruses infecting humans that are released in the bloodstream during a short period or during the whole length of infection can potentially be transmitted by blood or blood products. The majority of the emerging viral infections that are classified as a threat for blood transfusion are viruses with RNA genome, although some of them are DNA (Table 1). In terms of blood safety screening, transfusion-transmitted viruses can be categorized into major and minor agents. New agents have been added to the list of classical blood-borne viruses (Table 2).

Imported transfusion-transmitted viruses

Since most blood-borne, transfusion-transmitted viral infections are confined to endemic areas, the truth is that the borders have progressively become blurred, and it is currently difficult to establish clear patterns for screening based exclusively on country of origin.

Outbreaks of viral diseases outside endemic areas with local transmission events are currently common. This is the case for Dengue in Florida in 2009 and 2010[12] and Hawaii in 2011[13], West Nile in the USA since 1999[14], and Chikungunya in Italy in 2007[15]. The greatest health risk of arbovirus emergence comes from extensive tropical urbanization and the colonization of this expanding habitat by the highly anthropophilic mosquito, *Aedes aegypti*. In addition, the recent invasion into the Americas, Europe, and Africa by *Aedes albopictus*, an important vector for Chikungunya and Dengue, could enhance urban transmission of these viruses in tropical as well as in temperate regions[16]. In this scenario, during the viremic period in asymptomatic persons, there is a potential risk for transfusion-transmitted disease.

Dengue virus

Dengue is major public health problem throughout the tropics and subtropics. An estimated 50 million

Table 1. Main viral infections potentially transmissible by blood transfusion

Virus	Family/genus	Genome	Geographic area	Blood/organ-donation transmission	Obligatory testing	Antiviral therapy
HIV	*Retroviridae/Lentivirus*	RNA	Worldwide	Known/high	Yes	Antiretrovirals
HTLV	*Retroviridae/Deltaretrovirus*	RNA	Worldwide; high prevalence in Caribbean, Tropical Africa and Japan	Known/high*	No	No
HBV	*Hepadnaviridae/Orthohepadnavirus*	DNA	Worldwide	Known/high	Yes	PEG-INF; antivirals
HCV	*Flaviviridae/Hepacivirus*	RNA	Worldwide	Known/high	Yes	PEG-IFN + RBV and PI
HEV	*Hepeviridae/Hepevirus*	RNA	Worldwide	Theorical	No	Ribavirin
Dengue	*Flaviviridae/Flavivirus*	RNA	Latin America and Southeast Asia	Known/low	No	No
WNV	*Flaviviridae/Flavivirus*	RNA	Africa, Europe, the Middle East, west and central Asia, Oceania and North America	Known/low	Yes (only in USA)	No
Chikunguya	*Togaviridae/Alphavirus*	RNA	Central and South Africa, Southeast Asia, Australia, Italy and France	Theorical	No	No
Human parvovirus B19	*Parvoviridae/Erythrovirus*	DNA	Worldwide	Known/low	No	No
vCJD	*Prion*	Prion	UK, USA and Europe	Known	No	No

HTLV: human T-cell lymphotropic virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HEV: hepatitis E virus; WNV: West Nile virus; vCJD: Creutzfeldt-Jakob variant; PEG-IFN: pegylated interferon; RBV: ribavirin; PI: protease inhibitor.

Table 2. Classification of blood-borne viruses

Major (obligatory testing):
– HIV
– Hepatitis B virus
– Hepatitis C virus

Minor (facultative testing):
– Human parvovirus B19
– Hepatitis A virus
– Cytomegalovirus
– Human T-cell lymphotropic virus
– West Nile virus
– Hepatitis E virus

cases occur annually and 40% of the world's population lives in areas where there is risk of DENV transmission[17,18]. This is transmitted from person to person through the bite of an infected mosquito (mainly *Aedes aegypti*, *Aedes albopictus* or less commonly *Aedes polynesiensis*). The DENV replicates in humans during an incubation period of 3-14 days before the onset of symptoms. Infected persons, even those who remain asymptomatic, have concentrations as high as 10^7 viral RNA copies/ml of blood and can transmit the virus to mosquitoes as early as 1-2 days before symptoms develop[19].

During the viremic period, DENV can become a blood-borne infection. Cases of dengue after receiving blood products or donor organs or tissue, and after occupational exposure in a healthcare setting have been reported[18,20]. DENV can be transmitted from mother to fetus *in utero* or to infants at delivery (perinatal transmission)[21].

Since DENV was identified as one of the three high-priority infectious agents with potential risk for transfusion transmission in the USA[8], the only approach to prevent transfusion transmission of DENV-positive blood donors is the screening with sensitive nucleic acid amplification tests (NAT). In non-endemic areas, asymptomatic infection is primarily associated with

travelers returning from dengue-endemic areas. Importation risk is likely to grow with emergence of dengue globally and ever-increasing international travel[18].

Chikungunya virus

Chikungunya fever is an acute febrile illness associated with arthritis and arthralgia, initially identified in Tanzania in the 1950s[16,22]. Retrospective assessment determined that the recent Chikungunya virus (CHIKV) outbreaks started in 2004 in Lamu, Kenya, where an estimated 13,500 persons (> 70% of the population) were infected[23]. The CHIKV infections spread to the Indian Ocean islands next, initially in the Comoros and then Rèunion, where 244,000 cases occurred by April 2006 in a population of 766,000; the estimated attack rate was 35%[24,25]. During the dramatic outbreaks in 2005-2006, imported chikungunya cases were initially reported in European travelers returning from the Indian Ocean islands[15,26]. Subsequently, imported CHIKV was reported globally. In July and August of 2007 an outbreak of autochthonous CHIKV infection occurred in northeastern Italy, with 205 confirmed cases associated with an index case from India[15]. This was the first autochthonous transmission in a temperate region, an alarming event because the vector, *Aedes albopictus*, is widespread in many regions of the USA and Europe[27,28]. A program against an eventual introduction of CHIKV in the Americas due to the widespread distribution of the vector across the continent has recently set up[29].

The incubation period for CHIKV infection is most commonly 3-7 days (range 2-12) and infection is asymptomatic in 3-25%[22]. Outbreaks of CHIKV in temperate regions could affect the blood supply. The CHIKV infections are associated with high viral titres, around 10^4 to 10^7 per ml[30]. The CHIKV outbreaks in Rèunion and Italy required a temporary halt to blood donations and importation of blood products[31].

West Nile virus

West Nile virus (WNV) is a single-stranded RNA virus positioned taxonomically within the Japanese encephalitis virus serocomplex in the genus *Flavivirus*[16]. The relevance of WNV in blood banks emerged after its introduction into New York City (NYC) in the summer of 1999[32]. The invading strain was closely related to a 1998 isolate from Israel that caused widespread illness in geese. Interestingly, the resulting NYC outbreak seemed to be amplified among house sparrows and was associated with high viremia and mortality in American crows[33]. Ten years after its recognition in NYC, more than 25,000 cases of WNV had been reported in humans, including over 1,000 deaths in the USA, Canada, Mexico, and both Central and South America[34]. A large outbreak has occurred during the 2012 summer in Dallas, Texas[35].

West Nile virus causes a self-limiting febrile illness in 20% of immunocompetent hosts. While only one out of 150 infected individuals (0.7%) may develop meningoencephalitis, this complication may increase to 40% in immunocompromised hosts[36].

The transmission of WNV between humans or from animals does not occur. The virus is transmitted primarily by the bite of the *Culex pipiens* mosquito, but can also be transmitted by blood transfusion and organ transplantation since viremia has been reported to persist for 2-3 months. However, relatively high levels of viremia in the absence of antibody rarely last more than two weeks, and this is the period during which an individual might be infectious via blood transfusion[7]. Blood donor screening was initiated in 2003, with mini-pool NAT. During the time of high WNV activity, typically from June to September, conversion from NAT mini-pool to individual unit screening is performed so as to reduce the false-negative rate from low viral load in donors[37]. All donors in North America are screened for WNV infection, whereas travelers from the USA are not considered for WNV screening in Europe.

Other arbovirus infections

Many other viruses may cause human outbreaks outside their usual location, such as Rift Valley fever virus, Blutongue virus, Venezuelan equine encephalitis virus, Zika virus, Mayaro virus, or St. Louis encephalitis virus[22]. Arboviruses have a well-documented history of emergence expanding beyond typical geographic niches, often through human transportation, and enhanced amplification in domestic animals leading to spillover to humans. Since all arboviruses exhibit a viremic period in the absence of symptoms, they should always be considered as a potential threat for blood safety.

Retroviral infections and simian foamy virus

HIV

HIV was the first transmissible virus to have a major impact on blood safety. Serologic screening was

implemented in 1985, leading to a dramatic decline in transfusion-transmitted HIV infections[38]. The introduction of NAT testing more than a decade later came closer to approaching zero risk, although sporadic reports of HIV transmission by blood transfusion in spite of NAT screening still occur[39-41]. The estimated current risk of transmission in the USA is 1/2,300,000[42], in sharp contrast to the rate in developing countries, where around 10% of new HIV infections result from transfusions of contaminated blood or blood products[43].

HIV-1 has a worldwide distribution, with high prevalence of non-B subtypes in sub-Saharan Africa and increasing proportions in North America and Europe[44,45]. Moreover, the emergence of new strains such as HIV-1 group P[46] and the continuous appearance of recombinant viruses pose a future threat[44]. The approved EIA (or ELISA) and Western Blot tests are able to detect HIV antibodies for all variants described to date, although some tests may give indeterminate results. In contrast, NAT tests only pick up the most prevalent HIV variants, and rare or new ones can be missed during the window period[40].

Human T-cell lymphotropic virus

Human T-cell lymphotropic virus (HTLV) types 1 and 2 are human retroviruses transmissible by blood transfusion and organ transplantation. Transmission of HTLV-1 with an infected organ may be associated with an increased risk of HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[47]. Given that the virus has null or minimal extracellular phase of free viremia in the bloodstream, HTLV cannot be transmitted through acellular products. This is why no cases of HTLV infection have been reported in hemophiliacs.

Infection with HTLV-1 is highly prevalent in Central and South America, several countries in sub-Saharan Africa, and in Japan. The number of HTLV-1 infections has been steadily increasing in Spain, mainly driven by immigrants coming from endemic areas[48]. Although there is no mandatory screening for HTLV in Spanish blood banks, many of them check for the presence of antibodies in special populations, such as donors from endemic regions. New variants of HTLV have been reported[49], although so far there is no evidence of human disease linked to HTLV-3 or HTLV-4 infection. The use of leukocyte-reduced products in addition to viral screening seems to be the major method for stopping transmission of HTLV infections.

Simian foamy virus

Reports of infection by simian foamy virus (SFV) in animal handlers a decade ago[50] highlighted that interspecies transmission of retroviruses must always be a concern. Although transmission of SFV by transfusion has been documented in experiments in nonhuman primates, transmission by transfusion has not been documented in humans so far. Moreover, there is no evidence of any human disease caused by SFV. However, in the absence of any further evidence, some regulatory agencies, such as in Canada, have decided on the permanent deferral of blood donations from individuals whose employment involves contact with monkeys or their body fluids[51].

Hepatitis viruses

Along with ABO reactions, posttransfusion hepatitis has been historically one of the most common complications of transfusion of blood and blood products. Although hepatitis B and C have been the most frequent agents by far, other hepatitis viruses are known to potentially be transmitted to blood recipients.

Hepatitis A and E

Due to their high resistance to inactivation procedures, non-enveloped viruses such as human parvovirus B19, human bocavirus, hepatitis A virus (HAV) and hepatitis E virus (HEV) pose a particular threat to blood products. However, transmission of HAV by transfusion is very rare, almost always from donors suffering an acute but asymptomatic episode of acute hepatitis A. As expected, this situation is mainly confined to regions endemic for hepatitis A. Given that HAV viremia does not last for more than a few weeks, the risk of donating contaminated blood is very low, even in these areas.

Hepatitis E is an emerging disease in industrialized countries. The HEV is a single-stranded RNA virus. Four genotypes (1-4) have been described, which differ in their worldwide distribution. The HEV genotypes 1 and 2 are restricted to humans and are hyperendemic in developing countries, where they are responsible for major waterborne outbreaks. In industrialized countries, hepatitis E is considered a travel-associated disease. However, infections caused by HEV genotypes 3 (Europe and North America) and 4 (Japan and China) are on the rise[52]. The HEV genotypes 3 and 4 are very close genetically and have been isolated from

humans and other mammalians (pigs, wild boars, and deer), suggesting zoonosis and/or food-borne transmission. Individuals with frequent contact with swine and other animals (e.g. slaughterers, veterinarians, and farmers) are those most frequently exposed to HEV. Transfusion cases have been reported in Japan, France, and the UK[53].

Although most cases of hepatitis E are self-limiting, chronic infections have been reported in transplant recipients and other immunodeficient populations such as HIV-infected individuals. Acute hepatitis E can be fatal in 1-3% of cases, with higher rates seen in pregnant women[52]. Chronic hepatitis E may result in cirrhosis. Using NAT screening by mini-pools, 13 cases were identified when testing 16,000 German donors (prevalence, 0.08%), all being HEV genotype 3. The overall seroprevalence of anti-HEV IgG amounted to 6%[54]. Another recent study has found that 10% of plasma pools derived from North America and Europe are positive for HEV RNA. Given that infected HEV donors are generally asymptomatic and antibody screening is not reliable for excluding viremic persons, NAT should be considered for blood bank safety, as is done for hepatitis B and C, HIV, and WNV[37].

Hepatitis B

Hepatitis B virus was identified more than 40 years ago and HBsAg testing has been part of blood screening since 1972. Although the sensitivity of serological assays has improved enormously over time, subjects during the window period may escape detection. Since the introduction of NAT, the residual risk of HBV transmission has declined dramatically[42].

The long-lasting persistence of HBV genomes in the liver (with detectable or undetectable HBV DNA in serum) in persons testing negative for HBsAg is termed occult HBV infection. The lack of HBsAg detection is rarely due to infection with variants (S-escape mutants) missed by commercial assays, being more frequently the result of infection with HBV variants strongly suppressed in their replication capacity[55]. The causes of HBV suppression in these individuals are not well understood, although the host immune surveillance and epigenetic mechanisms are likely involved.

Occult hepatitis B is a worldwide diffused entity. Controversy persists given the different sensitivity and specificity of distinct diagnostic methods. Almost all subjects with occult hepatitis B harbor anti-HBc and low-level serum HBV DNA, almost always < 1,000 IU/ml. These are individuals having recovered from HBV infection but unable to totally control viral replication. More importantly, occult hepatitis B can be transmitted (i.e. through blood transfusion and liver transplantation), causing classic forms of hepatitis B in newly infected individuals. The development of an immunosuppressive status (mainly by immunotherapy or chemotherapy) may induce HBV reactivation and development of acute and often severe hepatitis B. Occult hepatitis B may also favor the progression of liver fibrosis in chronic hepatitis C patients. Finally, occult hepatitis B may maintain most of the direct transforming properties of overt HBV infection, such as the capacity to integrate into the host genome and synthesize pro-oncogenic proteins, contributing to the development of liver cancer.

Hepatitis C

Since the implementation of NAT for HIV, HBV, and HCV in the USA in 1999, cases with negative HCV serology but positive nucleic acid detection have steadily declined in first-time donors[56]. The estimated incidence and residual risk of transmission for approximately 66 million units tested during the first 10 years is recorded in table 3.

Non-enveloped viruses

Infection by non-lipid membrane viruses continues to be a concern for persons with hemophilia and other recipients of blood products. While the inactivation of lipid-enveloped viruses such as HIV and HCV by solvents/detergents and/or heating now used in the manufacture of plasma derivatives is highly effective, such treatments are not sufficient to prevent the transmission of non-enveloped viruses such as human parvovirus B19 and V4[57].

Human parvovirus B19 is an ubiquitous, small non-enveloped DNA virus that infects up to 50% of persons by the age of 15 years. Most exposed children and young adults develop only a mild rash (erythema infectiosum), and serious complications are rare. However, infection during pregnancy can cause fetal death. In addition, human parvovirus B19 infection can produce severe anemia in persons with hemolytic anemias. Finally, acute infection may produce joint swelling in as many as 3-10% of children and up to 50-60% of adults, particularly women. The relatively high prevalence of B19V infection in the general population along with the large number of blood donations used in the manufacture of plasma-derived factor concentrates virtually ensures universal exposure of hemophiliacs[58]. In 2000, manufacturers

Table 3. Estimated incidence and residual risk of major transfusion-transmitted viral infections in USA donations

Virus	Incidence (per 10^5 person-years)	Infectious window (days)	Residual risk (per donated unit)
HIV	3.1	9.1	1 : 1,467,000
HBV	3.4	30-38	1 : 280,000 to 1 : 357,000
HCV	5.1	7.4	1 : 1,149,000

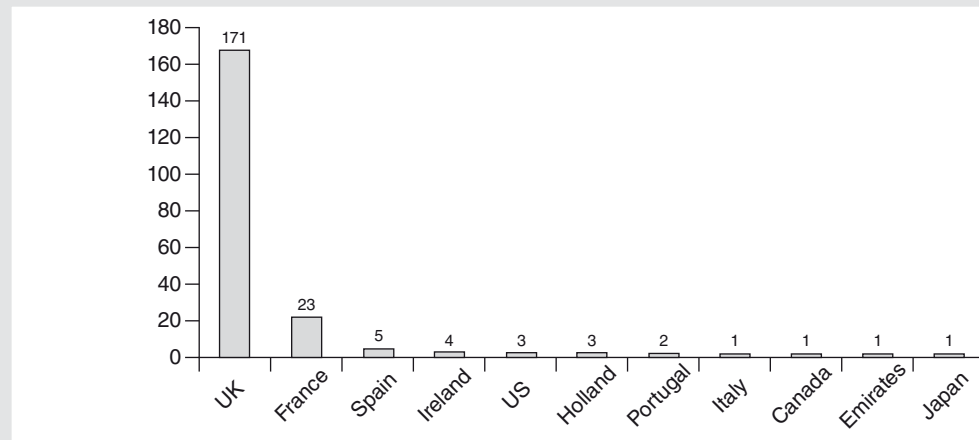HBV: hepatitis B virus; HCV: hepatitis C virus.

Figure 1. *Number of human cases of Creutzfeldt-Jakob variant reported up to 2010.*

began voluntary "in-process" NAT screening of plasma pools for B19V to improve the safety of the source plasma and solvent/detergent-treated pooled plasma[59].

A new erythrovirus has been recently identified in the plasma of HIV-infected individuals who acquired infection through injecting drug use[60]. It has been termed parvovirus 4 (PARV4). It is genetically distinct from existing genera within the family *Parvoviridae,* although viruses showing 61-63% sequence similarity to PARV4 have since been described in pigs and cows. Numerous studies have reported PARV4 DNA in human plasma used for transfusion, in plasma pools used for producing blood derivatives, and in purified coagulation factors. PARV4 viremia, while typically low, can reach levels as high as 5×10^8 virions/ml during acute infection.

Creutzfeldt-Jakob disease variant and prions

Creutzfeldt-Jakob variant (vCJD) was first recognized in 1995 as the human form of bovine spongiform encephalopathy (BSE), which became epidemic in British cattle in the 1980s. Both BSE and vCJD are transmissible spongiform encephalopathies, a family of animal and human neurological diseases thought to be mediated by prions[61]. Prions are cell-membrane proteins of unknown function. After years of incubation, vCJD manifests as a fatal neurodegenerative disease. Humans usually acquired vCJD by consumption of infected beef. Up to 2010, a total of 215 human cases had been communicated (Fig. 1), 171 of them in the UK[62].

Epidemiological studies of the vCJD in Britain revealed that three of them had received non-leukoreduced red blood cells 6-8 years previously from donors who developed symptoms after donations[63]. In addition, vCJD prions were also found in a hemophiliac treated with factor VIII concentrates, showing that blood derivates can also be infectious. Interventions used in the USA to prevent vCJD transfusion transmission include deferral for travel to, or residence in, the UK and Western Europe, or for receipt of transfusion in the UK or France[63]. Although tests for diagnosing vCJD infection in blood are being developed, the most

promising strategy appears to be prion removal using affinity filters in combination with leukoreduction.

Future prospects for safer blood components

Blood transfusion has become safer since the introduction of screening for most important viral infections. The WHO recommends that, at a minimum, blood be screened for HIV, hepatitis B, hepatitis C, and syphilis[64]. However, of 148 countries that provided WHO data for screening, 41 reported that they were not able to screen all donated blood for one or more of these infections. Of the 40 countries in sub-Saharan Africa, 28 have yet to implement national quality systems needed to assure effective screening of donated blood. The WHO estimates that the lack of effective screening results in up to 16 million new infections with hepatitis B, five million new infections with hepatitis C, and 160,000 cases of HIV infection every year. Overall, 5-10% of HIV infections worldwide are the result of transfusions of contaminated blood or blood products[43,64,65].

To increase access to blood transfusions and to promote blood safety, the WHO has for many years worked to help nations adopt an integrated approach for blood safety that has four key elements: establishment of a nationally coordinated blood transfusion service, collection of blood from exclusively voluntary donors from low-risk populations, testing of blood for compatibility and transfusion-transmissible infections, and reduction of unnecessary transfusions[65]. However, the continuous emergence of new infectious agents and the resurgence of old pathogens create crucial dilemmas for blood transfusion services. Clearly, the implementation of new technologies for screening, pathogen inactivation, and use of safer recombinant products is warranted for persons with bleeding disorders as well as for any with an urgent need for blood transfusion or blood derivates.

New screening tools

Periodic updating of exclusion questionnaires

The blood supply has become safer than ever, thanks to continuing improvements in donor selection and testing, along with broad improvements in public health. The first step in reducing the risk of transmission of infectious diseases through blood is to select voluntary non-remunerated donors from low-risk populations who give blood on a regular basis as these individuals are at a lower risk of transmitting transfusion-transmissible infections. Exclusion questionnaires have been the most useful tools to detect donors at risk for transmission of infectious agents. However, due to the evolving threats of emerging infectious diseases, questionnaires should be updated periodically following epidemiological alerts and information on travel to risky areas.

New nucleic acid test technologies

The introduction of NAT in 2000 for HCV, then HIV-1 in 2003, was finally followed by HBV in 2006. The previously accepted strategy of testing pools of various sizes was finally discarded, given the evidence of residual risk of transmitted-transfusion viruses using large pools[66]. The window period was shown to be a limiting factor for maximal efficacy of NAT, especially when applied to HBV or viruses with a relatively low peak of viremia, such as WNV.

Multi-pathogen microarrays and nanotechnology

Advances in biotechnology will continue to grow in the near future. The development of fully automated multiplexing assays for the simultaneous detection of several pathogens in microarray plates or using nanotechnology will most likely facilitate the development of new generation instruments ready to be used in clinical laboratories[67].

Pathogen inactivation

Dedicated viral inactivation treatments are a cornerstone in ensuring a sufficient safety margin to biologicals. Most techniques are effective for the inactivation of lipid-enveloped viruses, whereas non-enveloped viruses are more stable and the methods less effective for inactivation (Table 4).

One of the most efficient strategies to prevent blood-borne transmission of infectious agents is to develop and implement strategies for universal pathogen inactivation that would not only reduce or eliminate the risk of known pathogens, but would preemptively destroy any significant pathogen that might emerge in the future.

The concept of physiochemical pathogen reduction has been in development for the past two decades. The greatest success thus far has been in the use of solvent/detergent combinations to destroy enveloped

Table 4. Major pathogen reduction methods for blood components and their limitations

Agent	Plasma	Platelets	RBC	Limitations
Solvent-detergent (SD)	Yes	NA	NA	SD does not inactivate non-enveloped viruses (HAV or human parvovirus B19)
Methylene blue (MB)	Yes	NA	NA	MB does not effectively permeate cell membranes and intracellular pathogens may not be inactivated
Psoralen	Yes	Yes	NA	Urticaria is frequently experienced by patients treated for coagulation deficiencies
Riboflavin	Yes	Yes	UD	ND
Ultraviolet light	NA	UD	No	Large-scale investigation is needed to determine their efficiency and reliability
Alkylating agent	NA	NA	UD	Antibodies to RBC neoantigens
Nanophiltration	Yes	ND	Yes	13% of the RBC hemoglobin content is lost in the filtration process

Yes: approved in various countries; NA: not applicable; ND: not determined; UD: under development; HAV: hepatitis A virus; RBC: red blood cell.

Table 5. Infectivity reduction after the use of pathogen inactivation systems for the major blood-borne viral transmission

Virus	Genome	Enveloped	Size (nm)	Transmitted by		Infectivity reduction (log)
				Plasma	Cellular	
HIV-1/2	ss-RNA	Yes	80-100	Yes	Yes	> 6.2 cell free
HTLV-1/2	ss-RNA	Yes	80-100	No	Yes	4.7-5.1 cell associated
HBV	ds-DNA	Yes	40-48	Yes	Yes	> 5.5
HCV	ss-RNA	Yes	40-50	Yes	Yes	> 4.5
WNV	ss-RNA	Yes	40-60	Yes	Yes	> 6
Parvovirus B19	ss-DNA	No	18-26	Yes	Yes	4.0-4.9
HEV	ss-RNA	No	35-39	?	?	?
vCJD	Prion	No	10	Yes?	Yes	3 logs

HTLV: human T-cell lymphotropic virus; HBV: hepatitis B virus; HCV: hepatitis C virus; WNV: West Nile virus; HEV: hepatitis E virus; vCJD: Creutzfeldt-Jakob variant; ss: single strand: ds: double strand.

viruses, including HIV, HTLV, and HCV[40]. However, some agents exhibit intrinsic resistance to pathogen inactivation processes. This is the case for prions, some non-enveloped viruses (e.g. HAV or HEV), and bacterial spores. Furthermore, extraordinarily high titers of viruses in the bloodstream, as occasionally occurs with human parvovirus B19 or HBV, may result in inactivation failures below an infectious dose (Table 5)[8].

The prized goal of blood safety would be a method to inactivate all viruses regardless of their nucleic acid and protein structure, as well as bacteria, parasites, and any replicating structure.

Nano filtration

The development and availability of biocompatible viral filtration systems (also known as nano filtration), using membranes of a pore size as small as 15 nm, has improved the removal of infectious agents over recent years. These systems are specifically designed to remove, depending upon membrane used, typically > 4-6 logs of virus under conditions ensuring good protein permeability and recovery[68]. Validation studies and production experience throughout the world have demonstrated that viral nano filtration is a robust and

reliable viral reduction technique that can be applied to essentially all biological products.

Synthetic and recombinant blood products

There is a continuous demand for blood making, as it is increasingly difficult to achieve a large supply of donors. This fact has encouraged the development of surrogates for human donated blood. Ongoing efforts are in place to produce "artificial blood components", such as red blood cells, platelets, and also neutrophils[69]. Artificial blood may carry several advantages over donated blood, including larger supply, lower risk of blood-borne pathogen transmission, lack of risk for immune incompatibility, and extended survival of stored components.

Again, hemophilia care has been in the top line for the development of safer blood derivates[10,70]. Several plasma proteins artificially produced by recombinant DNA technology (e.g. activated factor VII, factor VIII, factor IX, activated protein C, thrombin, anti-thrombin, soluble thrombomodulin, tissue factor pathway inhibitor, and tissue plasminogen activator) have also been produced or are under development (e.g. von Willebrand factor, albumin, and immunoglobulins)[71]. In order to manufacture recombinant coagulation factors, genes (or modified genes) are inserted into cell lines. The cells are cultured and the secreted factor is purified from the culture medium[72]. Concern has been expressed over the use of human and animal products in the culture media, and over the use of human albumin as a stabilizer. If mouse monoclonal antibodies are used during the purification process, trace amounts may appear in the final product. Moreover, there is also the possibility of viral infection of cell lines used to produce the coagulation factor and any monoclonal antibodies. Even if all animal and human proteins can be removed from the production process, a further viral inactivation/removal step will enhance safety. For instance, moroctocog α, a last generation of recombinant factor VIII, is the only one which is purified with a synthetic peptide ligand and also incorporates a nanofiltration process to the solvent detergent pathogen inactivation step[73]. With all these procedures in place, the potential for removing infectious agents is extraordinary and the residual risk of infection is extremely low.

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