The Safety of Blood and Components, where is the limit?
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The recent past

HIV transmission by transfusion of blood and blood products was the biggest driver of change in the history of transfusion medicine in the past 30 years. Most of the medical world in the 70's and in the early 80's was celebrating the control of infectious diseases. The hemophilia community was celebrating the enjoyment of a normal life provided by the prophylactic use of plasma derived clotting factor concentrates. In this environment of excitement and trust in the power of medical science and pharmaceuticals, the initial reactions to the news that AIDS could possibly be transmitted by transfusion were denial and disbelief. The first report suggesting that clotting factor concentrates might transmit HIV was published in July 1982. It described three hemophilia patients who developed immunosuppression and opportunistic infections. By December 1982, four more cases had been identified among patients with hemophilia A (1). The first case of suspected transmission of AIDS by blood transfusion was reported at that time. Both the medical community and the patient community had no choice but to accept the growing evidence that AIDS was transmitted by a blood borne infectious agent. The epidemic ravaged through the United States, progressing from 1,000 recorded cases in February 1983 to over 500,000 cases in December 1995. In the early 80's, over 1% of blood donors in San Francisco, California, were suspected to be infected.

The Present

HIV was identified as the etiological agent of AIDS in 1984, and in 1985 screening assays for antibodies to HIV became available, leading to a remarkable reduction of the transmission of the infection by transfusion and transplantation (Table 1). This discovery was followed by substantial advances on serological and molecular screening for other transfusion transmissible viruses (HBV, HCV, HTLV-I/II, WNV), implementation of good manufacturing practices and quality assurance for blood centers and developments in computer systems that ensured accuracy of management of donors and donations. Unfortunately, despite preventive measures and substantial therapeutic advances, there are today over a 1 million HIV infected individuals in the U.S. and over 50,000 new cases are added each year (2). Europe continues to have a lower but still significant HIV incidence rate while the incidence in Asia has been high.

Virus	U.S.	Europe*
HIV	1:2,135,000	1:909,000 – 5,500,000
HCV	1:1,930,000	1:2,000,000 – 4,000,000
HBV	1:277,000	1:72,000 – 1,100,000
WNV	1:350,000	No reported cases
HTLV-I/II	1:2,993,000	Not tested

* Range between high and low endemic areas
Adapted from (3)

Despite of steady incidence rates of these infections in the general population, the number of reported cases of transmission HIV, HBV and HCV by transfusion has been extremely small in the U.S., in Europe and in many other countries. However, transfusion transmission continues to be a serious problem in countries with limited resources to invest in healthcare systems as reflected by the close correlation between the quality of blood transfusion services and the World Health Organization Human Development Index, or HDI (4). The Human Development Index classifies countries as having a low, medium or high HDI, based on life expectancy, educational attainment and adjusted income.

The Concept of Emerging Infections

The Institute of Medicine the U.S. National Academy of Sciences defined emergent infectious diseases as diseases of infectious origin whose incidence in humans has increased in last the two decades, or threatens to increase the future (5). Those include not only newly recognized diseases like AIDS in the 80s but also

infectious diseases that are reemerging due to conditions that facilitated their spread, as the case with West Nile Virus (WNV), Dengue virus (DENV) and Chikungunya virus (CHIKV). Factors that facilitated emergence have been climatic changes, globalization of human activities, air travel, migration, etc.

The magnitude and the reality of the epidemic of AIDS have generated and still generate great concern about future threats, particularly among chronic recipients of blood and blood products. Justly, they are scared by the possibility that an unknown or poorly understood transmissible agent could cause as much devastation in the future as HIV did in 80s. While only few of the recognized emergent infections today constitute a significant threat to the security of the blood supply, transfusion medicine has applied substantial resources for the monitoring of infections where the transmission by transfusion was suspected. Examples are the Idiopathic CD4+ Lymphocytopenia (or AIDS Without HIV), the systemic infection of American soldiers who returned from the Persian Gulf in 1990 with Leishmania tropica, and SARS coronavirus, until extensive investigation documented that they were not transmitted by blood transfusion (6).

Risk perception in blood safety

Despite the impressive progress in blood safety observed in recent years, public concerns and fear continue to be important motivators for the implementation of additional measures, many attempting to reach an unattainable “zero risk”. The public and patient advocates reluctantly accept the risk associated with procedures and medications other areas of medicine but have difficulty accepting risks associated with blood, even when the benefits clearly exceed the risks. The classical publication of Slovic (7) provides a basis for understanding public responses and attempts to help improve risk communications with the public and with decision-makers. He indicates that both dread and knowledge drive the ultimate public perception of risk. Essentially, known risks with a low degree of dread (e.g. smoking, boating and skiing) are accepted by the public even in face of serious consequences while unknown or misunderstood risks are seen as unacceptable because of poor knowledge about the event and the high degree of dread (e.g. nuclear reactor accidents, radioactive waste and recombinant DNA technology). Dr. Slovic wisely concludes that “each side, expert and public, has something valid to contribute. Each side must respect the inside and intelligence of the other.”

The Precautionary Principle and Zero Risk

Dr. Slovic’s analyses also help us understand distortions of the interpretation of guiding principles like the precautionary principle. One of the primary foundations of the precautionary principle, and its globally accepted definitions, are stated in Principle #15 of the Rio Declaration of the "Earth Summit" of 1992: "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation" (8). The European Commission has addressed the question of applicability of the precautionary principle and emphasized that it should be proportional to the chosen level of protection including, where appropriate and feasible, an economic cost/benefit analysis (9). Unfortunately, the words “cost-effective” and “proportional” are frequently dropped from references to the principle, leading to the widespread confusion with zero risk.

Some Emerging Infections of Current Concern

This article will focus on some of the emerging infections that are likely to be transmitted by transfusion of blood and components, like West Nile Virus (WNV), Trypanosoma cruzi (T. cruzi), the agent of Chagas’ disease, DENV and CHIKV. It will not address vCJD, bacterial contamination, and non-infectious risks such as TRALI, TACO and hemolytic reactions.

West Nile Virus in the U.S.

The U.S. experienced the true emergence of a transfusion transmitted disease in 1999, when WNV was introduced in New York City. WNV is transmitted to humans primarily through mosquito bites, and the

outcome of infection depends on the age and immune status of the exposed individual. WNV was first identified in 1937 in Uganda, and has caused small epidemics in Africa, the Middle East and Eastern Europe for many years. Recently, cases of infection have been recognized in Italy. WNV has become endemic in the U.S. with reoccurring outbreaks for nine consecutive years. Infecting between 1.8 and 4.1 million people since 1999, WNV has caused 28,943 documented cases of human disease and 1,130 deaths reported to the CDC and became the most common cause of viral encephalitis in the country. Birds are the major amplifying host of WNV and have facilitated the spread of the infection to all states in the continental U.S.

In addition, several non-avian vertebrates including mammals, reptiles and rodents, can be infected by WNV and some species produce levels of viremia capable of infecting mosquitoes (10). Over 60 species of mosquitoes are able to transmit the virus (11). The explosive WNV spread in the North America suggested viral adaptation and prompted concerns about genetic variability that could potentially decrease the sensitivity of blood donor screening and diagnostic assays increasing the risk of transmission to blood recipients, affect viral pathogenesis, development of vaccines, and development of therapeutic agents. Recent studies documented an increase in the number of mutations in the full WNV genome from 0.18% in 2002 to 0.37% in 2005 when compared with the original strain isolated during the 1999 epidemic in New York City. Essentially, WNV has slowly diverged from precursor isolates as the geographic distribution expanded (12).

Human-to-human transmission by blood transfusion was identified in 2002 (13) stimulating the rapid development and implementation of nucleic acid tests (NAT) for blood donor screening under FDA-approved investigational new drug protocols in 2003. Retrospective studies identified 23 cases of WNV transmission by transfusion in 2002 associated with blood components from 16 donations, whose retention samples or retrieved plasma co-components individually tested produced reactive results for WNV-RNA using a research-based PCR assay. Implementation of blood screening in the U.S. has been a success and since 2003 has resulted in the interdiction of ~2,600 WNV NAT-reactive units and the prevention of ~2,600 to 7,800 potential transmissions by transfusion. After introduction of donor screening by NAT there were 6 confirmed cases in 2003, one in 2004 and none in 2005; however there were 2 confirmed cases in 2006. There are potential transmissions in 2008 which are still under investigation.

Infection by Trypanosoma cruzi (Chagas disease)

Chagas' disease was first described in 1909 by Carlos Chagas, a Brazilian physician. The acute form occurs in about 20% of the infected people and appears 20 to 40 days after the vector insect bite or a blood transfusion. It is characterized by fever, lymphadenopathy and hepatosplenomegaly, and rarely by pericarditis and disturbances of cardiac conduction. It can be quite severe or fatal in recipients with a debilitated immune system. Parasitemia is frequent. Approximately 20% of recipients of infected blood remain asymptomatic. Generally, the patients recover totally after 6-8 weeks. The acute disease can be effectively treated with the experimental drugs nifurtimox or benznidazole.

The chronic form of Chagas' disease develops 10-20 years after the acute infection. Approximately 50% of the infected individuals have parasitemia without clinical symptoms. Since these individuals do not know that they are infected, they are accepted as blood donors. Approximately 20% develop a cardiopathy characterized by cardiomegaly, disturbances of conduction, and alterations of the electrocardiogram. The cardiac insufficiency is progressive and finally fatal. Between 9 and 14% of individuals chronically infected develop megaesophagus and megacolon as result of the disruption of myoneural junctions in the intestinal tract. In general, symptomatic patients have only one type of manifestations, cardiac or gastrointestinal.

Chagas' disease is caused by the protozoan flagellate *Trypanosoma cruzi.* Blood forms of the parasite can be seen in smears of peripheral blood when parasitemia is high. T. cruzi infects a large number of peri-domestic animals like cats, dogs, rats, skunks, armadillos, sloths, mice, rabbits, etc., are infected by T. cruzi and serve as natural reserves for the agent. The insect vectors are blood sucking reduviidea (kissing bugs in the U.S., barbeiros in Brazil and vinchugas in Spanish speaking countries). They nest in cracks in the walls of mud houses with thatched roofs in rural areas. The insects bite preferably at night and following the bite, defecate close to the wound. When scratching, the victim introduces the infected excrement into the

broken skin or carries it to the eye mucosa. Chagas' disease is endemic in Central and South America and in parts of Mexico. The number of chronic carriers of infection is estimated at 11 million individuals. These individuals became infected in rural areas but have often migrated to urban centers in Latin America, U.S. and Europe. The transmission of T. cruzi has been remarkably reduced in many rural areas of Latin America as a result of application of insecticides and improvement of rural habitations.

Presently, all blood donors in Brazil, Argentina and several other Latin American countries are screened by commercial ELISAs or by indirect immunofluorescence. There have been seven documented cases of disease of Chagas by transfusion of blood in the North America between 1987 and 2007. All the patients developed acute disease and myocarditis. Past studies have shown that the prevalence of antibodies to the T. cruzi between blood donors in the U.S. is low. The potential for the establishment and the dissemination of the disease of Chagas in U.S. or Europe seems to be low because living conditions do not favor establishment of the natural cycle. However, the potential for transfusion transmission in non-endemic areas exists (14).

A test for antibodies to *T. cruzi* was licensed by the U.S. Food and Drug Administration (FDA) in December, 2006. About two thirds of the American blood centers initiated universal testing of all blood donors, all the time, in January, 2007. In March 2007 the FDA's Blood Products Advisory Committee (BPAC) recommended that universal testing be continued for a period of about two years until sufficient data were accumulated before consideration of some form of selective screening as applies in other countries as for instance Spain.

In April 2009 BPAC reviewed possible testing strategies for *T. cruzi* infection in blood donors, including universal testing, testing donors once or twice, selective testing of specific donor groups or blood components, testing combined with donor questions related to the donor or their parents living in endemic areas, or testing donors visiting endemic areas. Panel members dismissed questioning of travelers indicating that it was not warranted because of the substantial decrease of incidence of T. cruzi infections in the endemic areas that has occurred in recent years as a result of control of reduvid vectors, and that infection occurs particularly in children after years of exposure in thatched houses where the vectors have nested.

The Committee voted to recommend that "<u>one</u> negative test would qualify a donor for all future donations without further testing or questions regarding risk of a newly acquired infection, subject to continuation studies to define the incidence of new infections in previously screened negative donors." The Committee did not consider selective testing based on questions about birthplace of the donor or the mother of the donor because studies presented at the meeting showed that questions had 75% sensitivity. It was clear that many donors gave inaccurate answers because of concerns about their immigration status. This recommendation came in the wake of draft guidance from FDA that suggested that all donations of blood or organs to be tested for antibodies to *Trypanosoma cruzi.* It is expected that FDA will accept the recommendations made by BPAC and issue a Final Guidance recommending selective testing (15).

Dengue and Chikungunya viruses in blood donations

Arbovirus epidemics are raging in tropical areas. Dengue virus (DENV), dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) affect millions of individuals every year and cause significant mortality in Latin America, Africa and Asia. CHIKV virus (CHIKV) has caused recurrent epidemics in the Indian subcontinent and recent epidemics in Reunion and other islands in the Indian Ocean, with recent arrival in areas of Europe. The surprising seriousness of recurring epidemics of WNV in North America has heightened concerns about the potential for introduction and similar epidemic spread of other arbovirus infections in the US. Dengue has received particular attention since cases have been recognized in the US at the border between Texas and Mexico. DENV is transmitted efficiently by the mosquito *Aedes egypti* and less efficiently by *Aedes albopictus.* CHIKV became well adapted to *Aedes albopictus*, the tiger mosquito after a single mutation in its genome described during outbreak in 2007 (16).

Despite the recognition of millions of cases of DENV infection and disease every year, there are very few published reports of transfusion transmission, one in Hong Kong, and another in Singapore. There are also

reports of transmission by needle sticks and one case associated with a bone marrow transplant in Puerto Rico. However, transmission by transfusion is often difficult to evaluate in the midst of an epidemic because the infection could have been acquired through a mosquito bite, through a transfusion or even through a needle stick. These facts raise questions about appropriateness of development of precautionary measures to prevent transfusion transmitted DENV in non-endemic areas. Research screening tests for DENV have been developed and have been the subject of publications (17). These studies documented the presence of asymptomatic viremic donors in Honduras and Brazil that could theoretically transmit the virus to blood recipients.

Transmission of CHIKV by transfusion is probable, but has not been documented. The CHIKV epidemics that raged through Reunion Island in the Indian Ocean from 2005-2007 prompted the French government to suspend whole blood collections and provide the red blood cell needs from the mainland. Platelet collections by apheresis continued locally, but the collected products were subjected to a process of viral inactivation. It should be noted that here have been no reports of CHIKV transmission by transfusion despite estimates that over 300,000 people were infected during these epidemics.

What is the reason why the number of reports of TT of DENV and CHIKV are so few? There are many differences between these viruses and WNV, but they do not clearly explain the lack of transfusion transmission reports for DENV and CHIKV. WNV infects a large number of birds and mammals. Birds are highly efficient amplification hosts, presenting very high viremia. Many species of mosquitoes that transmit WNV bite both animals and humans.

DENV and CHIKV do not have an amplification host. Amplification occurs in the salivary glands of *Aedes aegypti* and *Aedes albopictus.* These mosquitoes transmit the viruses from human to human in densely populated areas. In addition, DENV and CHIKV epidemics currently occur primarily in developing countries. Large numbers of individuals are affected simultaneously, overwhelming hospital emergency rooms, making impossible accurate anamnesis, physical examination and appropriate reporting. The environment is not conducive to clinical studies, even observational, that could adequately document case reports, let alone estimate rates of transfusion transmission. During epidemics, blood is diverted to the many cases with Dengue hemorrhagic fever and Dengue Shock Syndrome.. Thus, many of the patients that receive blood transfusions during the height of the epidemic are already infected with dengue. Postponement of other hospital activities like elective surgeries reduces the opportunity of transmission of infection to naïve patients by transfusions. Lookback is rarely performed in developing countries because of limited resources.

The availability of potential donor screening assays for DENV and CHIKV RNA is welcome and reassuring. Available research assays could be quickly developed to address epidemics occurring in non-endemic areas. However, the need for implementation of donor screening assays for DENV or CHIKV is questionable. Epidemics in tropical areas affect tens of thousands of individuals and overwhelm the healthcare system. All resources and efforts are directed to the sick population. The value of implementation of donor screening or other high cost prevention measures to protect blood safety in those areas would require careful consideration, taking into account prevalence of viremia in donors, transmission rates, and disease penetrance in infected recipients (18).

It could be argued that screening assays for DENV and CHIKV would be beneficial for qualification of travelers to endemic areas as blood donors. The actual benefit of such screening is unclear because many of these potential donors would be deferred because of malarial risk. DENV and CHIKV are clear example of situations were the application of the Precautionary Principle should be carefully analyzed taken into account donor loss vs. blood safety. Would it be appropriate to steer some of the very limited resources available in countries with low HDI to a few blood recipients, in the absence of clear idea about the frequency of transmission of these viruses?

Finally, we hope that public health authorities, regulatory agencies, blood banking organizations, and manufacturers of products all support and invest in the development of technologies that may be useful for viral inactivation of all cellular components. Pathogen reduction is a more generic and proactive approach to address risks associated with arboviruses, precluding the need for implementation of donor screening

assays. The example of clearance of viruses by plasma fractionation and viral inactivation procedures is remarkable, and should encourage further pursuit of methodology applicable to cellular components. It would address WNV, DENV, CHIKV, T. cruzi, plasmodia, and other reemerging agents as yellow fever virus which is reappearing in South America both in wild monkeys and in humans, with several reported human deaths (19, 20).

References

1. Centers for Disease Control. Update on AIDS among patients with hemophilia A. MMWR 31:644-6 (1982
2. http://www.cdc.gov/mmwr/PDF/wk/mm5736.pdf, last accessed on April 15, 2009
3. Bihl F et al. *Journal of Translational Medicine* 2007, **5**:25 doi:10.1186/1479-5876-5-25 (http://www.translational-medicine.com/content/5/1/25), last accessed on April 15, 2009
4. http://www.who.int/bloodsafety/global_database/en/SumRep_English.pdf, last accessed on April 15, 2009
5. http://books.nap.edu/openbook.php?isbn=0309047412, last accessed on April 15, 2009
6. Bianco, C and Rios, M. HIV Transmission by blood transfusion. In "Blood Safety and Surveillance". Linden J.V. and Bianco, C., eds. Marcel Dekker, Inc., New York, pp 251–278, 2001
7. Slovic P. Perception of Risk. Science 236:280-285, 1987
8. http://www.unep.org/Documents.multilingual/Default.asp?DocumentID=78&ArticleID=1163, last accessed on April 15, 2009
9. http://ec.europa.eu/dgs/health_consumer/library/pub/pub07_en.pdf, last accessed on April 15, 2009
10. van der Meulen KM et al. West Nile virus in the vertebrate world Arch Virol (2005) 150: 637–657)
11. Gubler DJ. The Continuing Spread of West Nile Virus in the Western Hemisphere. Clin Inf Dis 45:1039–46, 2007.
12. Grinev A et al. Variability of West Nile Virus in US Blood Donors, 2002-2005. Emerg Infect Dis. 14:436-444, 2008
13. Pealer, LN, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 349:1236-45, 2003
14. Schmuniz, G.A. Trypanosoma cruzi, the etiologic agent of Chagas' disease: status in the blood supply in endemic and non-endemic countries. Transfusion, 31:547, 1991
15. http://www.fda.gov/ohrms/dockets/ac/09/briefing/2009-4428B1-3.htm, last accessed on April 15, 2009
16. Tsetsarkin KA et al. A Single Mutation in Chikungunya Virus Affects Vector Specificity and Epidemic Potential. PLoS Pathog 3(12): e201. doi:10.1371/journal.ppat.0030201, 2007.
17. Linnen J, et al. Dengue viremia in blood donors from Brazil, Honduras and Australia. Transfusion 48:1348-1354, 2008
18. Bianco C. Dengue and Chikungunya viruses in blood donations: risks to the safety of the blood supply? *Transfusion*, 48:1279-1281, 2008
16. Allain JP, Bianco C; Blajchman MA, Brecher et al. Protecting the blood supply from emerging pathogens: the role of pathogen reduction. *Transf Med Rev* 19:110-126, 2005
17.http://www.promedmail.org/pls/otn/f?p=2400:1001:2160992847569112::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1000,77042