VIRAL INFECTIONS TRANSMITTED
THROUGH TISSUE TRANSPLANTATION

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ABSTRACT

The incidence of tissue allograft-transmitted infection is unknown and can best be inferred from prospective studies – that have not yet been performed and reported. Viral infections have been transmitted via tissue allografts such as bone, skin, cornea, and heart valves. Bone allografts have transmitted hepatitis C, human immunodeficiency virus (HIV-1), and human T-cell leukaemia virus. Corneas have transmitted rabies, hepatitis B virus, cytomegalovirus (CMV), and herpes simplex virus. Heart valves have been implicated in transmitting hepatitis B. HIV-1 and CMV have been transmitted by skin allografts. Use of comprehensive donor eligibility criteria; excluding potential donors with behaviours risky for HIV-1 and hepatitis infection, and donor blood testing have greatly reduced the risk. Recent reports of HIV transmitted from a seronegative donors prompts the addition of viral nucleic acid testing of the donor. During tissue processing, many allografts are exposed to disinfectants and sterilisation steps such as gamma irradiation, which further reduce or remove the risk of transmitted disease. Some viruses are fairly resistant to gamma irradiation and the high doses needed may be harmful to the tissue allografts. Because the effectiveness of some tissue grafts depends on cellular viability, not all can be subjected to sterilisation steps, and, therefore, the risk of infectious disease transmission remains. For these, preventing the transmission of viral infection relies mostly on careful donor selection and viral testing, but processing with mild disinfectant can be useful. To further assure safety in the use of allografts, the physician and hospital should select tissue banks that follow national professional standards as their source for allografts.

INTRODUCTION

Tissue transplantation therapy, which has been utilised for over 50 years [1], is a rapidly developing field carrying with it great promise for ameliorating or curing many diseases. One of its drawbacks, however, is the potential for donor-to-recipient disease transmission. This risk is greatly reduced by excluding donors at risk of carrying infection and by testing the donor for transmissible infectious disease. Aseptic surgical technique in a quality environment, when removing the tissue from the donor, when processing and storing the tissue and during implantation is critically important to prevent bacterial and fungal contamination. Non-viable tissue grafts such as bone can undergo disinfection and sterilisation steps. During the past two decades the disease transmission risk associated with tissue transplantation has been greatly reduced by implementation of standards set by professional organisations, such as the American

Association of Tissue Banks [2], the European Association of Tissue Banks (EATB) [3], the Eye Bank Association of America [4], and governmental regulations. However, the incidence of transplant-transmitted infection is unknown and the studies needed to determine this have not been performed.

Cadaveric donations (Table 1) and clinical transplants (Table 2) of cornea, bone, skin, heart valve, and other tissue allografts in the USA greatly exceed that of organs [5]. Organ transplantation flourished in the early 1980s following the discovery and introduction of cyclosporin as an effective immune suppressant.

Table 1. Cadaveric organ tissue donation in the USA.

Type of Donor Tissue	Donations Per Year
Cornea donors *	46,729
Bone, skin, or other tissue donor †	18,021
Organ donor ‡	6,082

* Eye Bank Association of America (EBAA) [4]
† American Association of Tissue Banks (AATB) [2]
‡ United Network for Organ Sharing (UNOS) [5]

Table 2. Estimated number of allografts transplanted annually in the USA.

Tissue	Transplants
CADAVER TISSUE *	
Bone	675,370
Cornea †	50,868
Skin (sq. ft.)	11,222
Heart Valve	5,500
Vessels	433
Pericardium	5,327

* American Association of Tissue Banks (AATB) [2]; Office of the Inspector General [6]
† Eye Bank Association of America – 2001

This brought a large supply of cadaveric donors available that could also be used for tissue donation. Unlike the limitations of organ transplants, tissue transplantation generally is not limited by HLA histocompatibility barriers [7] or by ABO blood group

incompatibility [8]. No longer a scarce resource, the widened availability of tissue allografts encouraged new clinical use and brought attention not only to their effectiveness and advantages over autografts but also to their drawbacks, side effects, and complications. One complication of tissue transplantation has been transmission of viral infections of donor origin to the recipient [9,10]. Viral infections can be transmitted if the donor has a viral infection and the viral levels are too low for detection. In asymptomatic donors who are recently infected, a transient viremic phase can exist prior to development of a positive donor-screening test for antibodies. Preventing donor-to-recipient infectious disease transmission relies heavily on selecting safe donors not only through testing, but also by medical and social behaviour screening to select donors more likely to be free of transmissible infections. In addition, sterilisation with gamma irradiation can be important.

Some tissue allografts, e.g. corneas, heart valves and skin, need to remain viable and cannot be exposed to disinfectants or sterilised without an unacceptable loss of viability. Other grafts are non-viable, largely comprised of acellular connective tissue, and can be disinfected or sterilised more freely resulting in a greater assurance of sterility (Table 3). This review focuses on viral infections transmitted through transplanted tissues and steps taken for its prevention.

Table 3. Allograft characteristics affecting ability to transmit disease.

Nonviable Allograft	Viable Allograft
Type	
Bone	Heart valve and vessels
Dura mater	Cornea
Pericardium	Skin
Tendon	Marrow
Costal cartilage	Blood stem cells
Fascia	Vascularised organs
Ear ossicles	Semen and oocyte
	Foetal tissue
Characteristics	
Non-viable	Contains viable cells
Acellular	May be antibiotic treated
Connective tissue	Cannot be sterilised
Can be processed, sterilised	

VIRAL INFECTIONS TRANSMITTED BY CORNEAL ALLOGRAFT

Human Immunodeficiency Virus:

As thin avascular tissue, cornea comprises a well-hydrated transparent layer of connective tissue and a single-cell layer of viable endothelial cells. Consequently, it is not very immunogenic nor is it often rejected by the recipient, unless it becomes vascularised. Similarly it is not very efficient in transmitting viral infectious disease from the donor. Diseases transmitted through corneal transplants are listed in Table 4. The cornea is not efficient in carrying or transmitting HIV. Based on assumptions about HIV antibody test sensitivity, Goode *et al.* estimated that 3 per 10,000 cornea allografts would be from HIV-infected donors despite HIV antibody testing [11]. There have been several documented cases in which cadaveric organ and tissue donors were infected with HIV but the cornea recipients did not become infected. Although HIV has been isolated from tears, retina, cornea, aqueous humour, iris, and conjunctiva [12-16], HIV from infected cadaveric donors has not been transmitted to cornea recipients [17,18]. This should not be surprising because the inoculum of HIV is small in the relatively avascular, hypocellular cornea compared to that in an organ transplant or a blood transfusion from an HIV infected donor.

Table 4.   Viral diseases transmitted by tissue allografts.

Allograft	Virus
Bone	Hepatitis C
	Hepatitis, unspecified type
	HIV
	HTLV
Tendon	Hepatitis C
	HIV
Cornea	Hepatitis B
	Rabies
	Herpes simplex virus
	Cytomegalovirus (?)
Heart Valve	Hepatitis B
Skin	HIV (?)
	Cytomegalovirus (?)
	Hepatitis C(?)
Saphenous vein	Hepatitis C

Hepatitis B Virus

Failure to transmit viral infection from hepatitis B surface antigen (HBsAg)-positive donors has been reported in two recipients of corneas. This suggests that the cornea is inefficient as a mode of HBV transmission, although in these cases the administration of hepatitis B immune globulin and vaccine to the recipient may have prevented infection [19].

Khalil *et al.* assessed the presence of HBsAg and HBV DNA in corneal buttons taken from HBsAg-positive donors [20]. They found HBV in a small percentage of corneas. Others studied 31 donors infected with HBV or HCV and were unable to detect HBV DNA or HCV RNA in the corneas [21]. Despite this inefficiency, HBV transmission by corneal transplantation has been reported.

In earlier reviews by O'Day [22] and Raber and Friedman [19] there were brief reports of hepatitis B transmission to cornea recipients from HBsAg positive donors. Two cases of recipient HBV infection after transplants from two different HBsAg-positive donors were eventually reported [23]. Corneal donations took place from two donors; one in 1984 from an alcoholic man and one in 1985 from an injecting drug user. Tests for HBV were performed on the donor retrospectively after recipients developed HBV infections. Both donors were positive for HBsAg. Only one of the two recipients of corneas from each of the two donors developed symptomatic HBV infection.

The use of current professional standards and federal regulations would have prevented these cases since exclusion of donors with hepatitis risk behaviours and testing for HBsAg are now required.

Rabies

Rabies virus infection in humans is often found in the cornea. Because of this, a cornea impression test has been useful for early diagnosis [24]. Corneal allografts are also capable of transmitting rabies. Seven cases of fatal rabies transmission from cornea transplantation have been reported in the US, France, Thailand and India during 1979-1988 and in Iran in 1994 [25-31]. The first case involved a 39-year-old man in the US with ascending paralysis [26] and the second involved a donor in France who died from paraplegia, encephalitis and myocarditis [27].

In 1997 Javadi *et al.* and Gode and Bhide each reported rabies developing in two patients who received corneal transplants from the same donor [25,31]. Each of these cadaveric donors had an obvious acute neurological illness clinically consistent with rabies. National professional standards used by tissue banks today prohibit the use of these donors and would have prevented these cases of rabies transmission.

Other Viruses

Cytomegalovirus does not appear to be readily transmitted by cornea transplantation from seropositive cadaveric donors to seronegative recipients. Of 25 seronegative patients receiving a corneal graft from a seropositive donor, only two seroconverted [32].

Herpes simplex virus, type 1, has been found widespread in corneal stromal cells but only one case of transmission by a cornea allograft has been reported. The infection caused corneal deterioration in the recipient by the fifth day after transplant [33]. HSV DNA was found in two of five cornea allografts from other donors [34].

VIRAL INFECTIONS TRANSMITTED BY BONE ALLOGRAFT

Hepatitis

Hepatitis has been reported from use of unprocessed refrigerated and frozen bone allografts, but not from bone grafts that were cleaned of cells and fat with water jetting and ethanol soaks prior to being freeze dried or treated with sterilants such as gamma irradiation or ethylene oxide. In 1954, prior to the availability of viral hepatitis testing of donors, a Yale medical student received a refrigerated bone graft to treat a depressed fracture of the proximal tibia and developed hepatitis with jaundice 10 weeks later [35]. The bone graft was obtained from the amputated leg of a patient with occlusive vascular disease and gangrene. Otherwise, the donor was in good health, with normal liver function tests and without a history of jaundice or liver disease. The donor had received blood transfusions 3 years previously.

Three reports from nearly a decade ago documented that hepatitis C virus (HCV) can be transmitted from donor-to-recipient through the use of frozen, unprocessed bone allografts [36,37,38]. In the first case, donor testing for HCV antibodies was not available. HCV was transmitted by the use of a femoral head allograft after it was donated by a donor undergoing hip arthroplasty and stored frozen for 2 months [36]. In a second report, HCV was transmitted from an infected cadaveric tissue donor through frozen, unprocessed bone and tendon grafts, but not through freeze-dried bone allografts that were treated with gamma irradiation [38]. In this study, the cadaver bone donor tested negative for HCV antibodies using the first generation test available at the time of donation in 1990, but stored serum tested positive when a new, more sensitive test was introduced in 1992. Testing for HCV RNA by polymerase chain reaction (PCR) was also positive. In a third brief report involving five HCV-infected organ and tissue cadaveric donors, a minority of the recipients of frozen bone allografts became infected with HCV [37].

In a more recent case, an HCV-infected organ and tissue donor was tested and found negative for HCV antibodies [39,40]. Despite this negative screening test for HCV, several organ and tissue recipients became infected. When blood samples from the donor were tested later for HCV RNA, the results were positive and this confirmed the link between the donor and multiple infected recipients. The donor had been recently infected and was viremic but had not yet produced detectable antibodies. Bone allografts from the same donor that had been treated with gamma irradiation did not transmit HCV. With the implementation of HCV RNA as a donor-screening test in the future, cases such as this would be prevented.

There have been no reports of HBV transmission through bone transplantation, although it has been recognised as a complication of organ, cornea, and heart valve transplantation. It is quite probable that there have been transmissions but none have been recognised and published.

Human Immunodeficiency Virus

HIV-1 has been transmitted through blood, tissues, and organs [41,42]. Viable HIV-1 can be recovered from bone, marrow, and tendons of patients with acquired immunodeficiency syndrome [43-46]. In 1984, a fatal HIV-1 infection was transmitted to a woman undergoing spinal fusion for scoliosis through the use of a frozen femoral head allograft several weeks after it had been donated during hip arthroplasty from a donor who had a history of intravenous drug abuse and who had an enlarged lymph node that

had been biopsied the previous year [47]. Both the donor and the bone allograft recipient subsequently died of AIDS. A test for HIV-1 antibody was not available at the time of donation. This donor would not have been eligible to be a donor today due to his history of intravenous drug abuse and lymphadenopathy. There have been other cases of HIV infection in recipients of bone allograft derived from HIV-infected donors who were not tested for HIV at the time of donation. Prior to HIV antibody test availability in Germany, 12 recipients had frozen bone allografts from an infected cadaveric donor during November 1984 through January 1985 [48]. Only four of these recipients became HIV positive. Seven remained HIV-negative. In Taiwan a man donated a femoral head during hip replacement surgery but was not tested for HIV. The bone allograft was used in a 34-year-old woman in 1996 during knee reconstructive surgery. She seroconverted with HIV antibodies when tested five months later [49].

Another reported case of HIV transmission through the use of frozen bone allograft involved a seronegative but infected cadaveric donor but the test was new and not very sensitive. Multiple organs, corneas, bones, and connective tissues were transplanted [42]. Three organ recipients and three recipients of frozen bone and tendon allografts became HIV infected. These allografts had not been sterilised with gamma irradiation or ethylene oxide gas prior to use. The donor tested negative for HIV antibody at the time of donation in October 1985, which was a few months after the first, relatively insensitive HIV antibody testing kits became available. Between 1985 and 1991, there were several modifications that greatly improved test sensitivity. Prior to 1989, HIV antibody was detectable a median of 63 days after initial infection [50,51]. A study of HIV infected blood donors between March 1987 and 1991, when whole viral lysate enzyme immunoassays were used to detect HIV antibodies, showed an average seronegative window period of 45 days [52]. A report in 1992 showed HIV antibody test kits in use at that time detected twice as many infected individuals as did the test kits available in 1985 [53]. Since 1992, HIV antibody tests became even more sensitive, detecting IgM, the earliest form of antibodies, an average of 8-20 days earlier [54,55] and resulting in a seronegative window period of approximately 22 days [56,57]. Since then blood donor testing for HIV RNA by nucleic acid testing (NAT) has been implemented and has further reduced the risk of a transfusion and when validated and implemented for cadaver tissue donors will reduce the risk in tissue transplantation [58].

The prevalence of HIV antibodies in bone donors is low and when the medical history screening and selection processes are applied vigorously, it should not be greatly different from that of voluntary blood donors. This may be true for living bone donors [59-61] but not necessarily for cadaveric donors. Of 9000 living bone donors who donated femoral heads at the time of hip arthroplasty surgery, none were found to have confirmed positive tests for HIV-1 antibodies at the time of donation [59]. Prevalence of infectious disease markers in surgical bone donors was not different from that of blood donors, except for a higher prevalence of false positive syphilis tests and antibodies to HBV core protein [60]. Retesting of 1608 living bone donors 180 days later yielded none with confirmed positive HIV or HCV tests [61]. Of 5513 cadaver bone donors tested throughout the United States in 1992, there were three confirmed positive for HIV antibodies [62], but these three were from a single tissue bank that later disclosed accepting donors with risk factors for HIV. A more recent survey by AATB [63] revealed a higher prevalence rate of infectious disease markers than have been reported for blood donors, with rates ranging from 2 to 40 times higher (Table 5). The addition of viral nucleic acid testing, since 1999, in screening blood donors for HIV and HCV has further reduced the risk from blood donors. First time blood donors have a higher infectious disease marker rate and may be more similar to organ and tissue donors [64].

Because prospective tissue donors with HIV risk behaviours and positive tests for HIV are excluded and most bone graft processing removes blood and marrow cells and applies disinfectants and sterilants, the risk of HIV transmission by bone transplantation is now very remote, if not nearly absent [65]. The risk of transmitting HIV through bone grafting has been calculated to be less than one in a million grafts [66,67], and is even less if the graft has been subjected to processing and sterilisation steps using gamma irradiation or ethylene oxide. However, the HIV transmission risk is higher in the less frequently used frozen unprocessed bone allograft. An accurate estimate of the risk cannot be made until a more accurate determination of the prevalence of HIV infection in the donor and recipient population is available and prospective studies have been done on recipients.

Table 5. Donor exclusions for hepatitis and HIV risk behaviour.

HIV and Hepatitis Risk Behaviour Exclusions

Persons with clinical or laboratory evidence of HIV infection

Men who have had sex with another man even one time in past 5 years

Non-medical injections of drugs in past 5 years

Persons with haemophilia or related clotting disorders who have received human-derived clotting factor concentrates

Persons who engaged in sex for money or drugs in past 5 years

Persons who have had sex with any of the above in past 12 months

Exposure to blood suspected to be HIV or hepatitis infected through percutaneous inoculation of open-wound or mucous membrane contact in past 12 months

Inmates of prisons for at least 7 days in past 12 months

Tattoo in past 12 months

Human T-Lymphotrophic Virus

Asymptomatic HTLV-I infection has been transmitted by transplantation of a fresh-frozen unprocessed femoral head bone allograft [68]. A 62-year-old man became infected by HTLV from a blood transfusion in 1987 during hip surgery. One month later he developed fever a rash and a transient right-sided radial nerve palsy. Frozen sera obtained during this illness but tested later demonstrated HTLV seroconversion. In 1991 he donated a femoral head without anti-HTLV testing during a second surgery for a hip prosthesis. The unprocessed frozen femoral head was used as a graft in a different patient one month later. This bone graft recipient developed HTLV-I antibodies but had no HTLV-I associated disease.

INFECTIONS FROM CARTILAGE AND OSTEOCHONDRAL ALLOGRAFTS

Costal cartilage allografts are routinely disinfected or sterilised prior to their use as allografts and provided in a freeze-dried or frozen form. There have been no reports of processed costal cartilage transmitting infection from the donor to the recipient. Donald and Cole surveyed 312 surgeons who used cartilage allografts preserved by 8 different methods for facial reconstructive surgery [69]. They found a postoperative bacterial infection rate of 19% that was similar to the 16% reported following use of autologous cartilage.

VIRAL INFECTIONS TRANSMITTED BY TENDON ALLOGRAFTS

Viral Diseases

The use of the patellar tendon allograft to replace the knee's injured anterior cruciate ligament has become commonplace [70]. HIV has been isolated from tendons in HIV infected persons [43,71] and has been transmitted from a seronegative cadaveric tissue donor through a donated patellar tendon used in knee surgery [42]. HCV was transmitted to recipients of frozen tendon allografts from an anti-HCV positive cadaveric donor [38]. It is possible that HIV and HCV were harboured in the unprocessed bone blocks at either end of the tendon allograft. These allografts had not been processed to remove blood and marrow cells. Despite these cases, the risk to recipients is presumably low as long as donor-screening steps are applied as required by national standards [2,72] and federal regulations [73-75]. In addition, tendons can be treated with gamma irradiation to further reduce the risk of disease transmission.

Selecting donors without risk factors and without HCV antibodies make the risk of spreading HCV by transplant an exceedingly rare event. However, an early HCV infection in a cadaveric organ and tissue donor not yet producing antibodies was reported recently [39,40]. A patellar tendon allograft recipient developed acute, symptomatic hepatitis C in May 2002, six weeks after transplantation. No other potential sources of infection were identified. The tissue donor was anti-HCV negative but stored serum showed HCV RNA when tested later. Thirty-nine other persons received tissues or organs from this same donor. Early results of a partially completed investigation showed that of 18 recipients tested, six showed HCV infection including a lung recipient who became HCV RNA positive on day 4 and died of liver failure 14 months later. Presumably, the cadaveric donor was in a viremic stage early in infection prior to antibody development. To date cadaveric testing for HCV RNA is not available for routine use. HCV RNA should be considered for cadaveric tissue donors as soon as test reliability has been evaluated particularly using cadaveric samples obtained up to 24 hours after death.

VIRAL INFECTIONS TRANSMITTED BY CARDIOVASCULAR ALLOGRAFTS

Viral Diseases

The capacity of human heart valve allografts to transmit HBV was demonstrated in a study of thirty-one patients who received heart valve allografts from HBsAg-positive donors. Twenty-two recipients were HBsAg-positive prior to transplant or were immune to HBV and not susceptible to HBV infection. Of the nine recipients

susceptible to HBV infection, only one developed HBV viral markers. None developed clinically apparent hepatitis. However, four susceptible recipients received hepatitis B immune globulin and one received HBV vaccine following transplant, which may have prevented infection [76]. Currently all donors are tested for HBsAg and if positive are excluded. Despite testing donors for HBsAg and anti-HBc, HBV transmission can still occur because some donors can have circulating HBV at levels not detectable in routine tests. Thijssen et al. found one of 676 heart valve allograft donors to have HBsAg detectable with routine screening tests [77]. In addition, they found 10% to have anti-HBc. Fifty-two of 63 donors with anti-HBc also had antibodies to HBV surface protein (anti-HBs), indicating a resolved HBV infection and a recovered, immune non-infectious status. Three of those with anti-HBc but without anti-HBs were positive for HBV DNA using a more sensitive liquid-phase DNA hybridisation assay. This would suggest a possible value of anti-HBc donor testing to prevent transmission of HBV; however, one study of blood donors has shown a lack of predictive value in preventing post-transfusion hepatitis [78]. More recently, however, several reports have confirmed that some anti-HBc positive donors will be positive for DNA and will transmit HBV [79].

Recently a case of HIV infection transmitted by use of a saphenous vein allograft was reported. The cadaveric donor had no known HIV risk factors or signs of hepatitis and had negative tests for HIV antibodies. Subsequent studies demonstrated HCV RNA in the donor serum that was the same serotype, 1a, as that found in the donor.

VIRAL INFECTIONS TRANSMITTED BY SKIN ALLOGRAFTS

Viral Infection

Viral disease transmission by skin allografts has been reported. Epidermal cells can be infected with HIV-1 and the epidermis of HIV-infected individuals can transmit HIV to white cells in vitro [80,81]. In one study HIV RNA was found in only one of twelve infected patients [82]. Clarke reported, in a brief letter, a weakly positive test for antibody to HIV-1 in a burn patient after receiving skin from an HIV-positive donor [83]. The results of donor testing were not known before the skin was used. The authors did not report whether other recipient risk factors were present or the results of confirmation testing. HIV transmission from skin allograft has been recently reviewed [84,85].

Transmission of hepatitis from skin allograft has not been reported although HCV nucleic acid has been demonstrated in skin from infected donors [38]. There are recent reports implying transmissibility of human cytomegalovirus (CMV). Animal models clearly demonstrate that skin grafts are capable of transmitting CMV [86-89]. Earlier studies by Kealey et al. showed that burn patients acquire CMV during hospitalisation and that blood transfusions may be a contributing factor [90]. A subsequent study eliminated blood transfusion as a contributor by studying patients who received skin allografts but no CMV-positive blood. They showed that CMV-negative burn patients who receive skin allografts from CMV-positive donors can seroconvert to become CMV-positive [91].

CMV resides in peripheral blood leukocytes in asymptomatic CMV antibody-positive donors long after their initial infection. Asymptomatic CMV-positive donors can transmit CMV infection through transfusion and transplantation if the recipient is CMV negative. Most healthy adult prospective donors have CMV antibodies, and therefore excluding CMV positive donors to prevent CMV transmission would exacerbate the already existing shortage of skin allografts and would not be practical. Testing donors for CMV antibody is not required by national professional standards.

Immunosuppressed individuals such as organ recipients have a high mortality and morbidity rate from transplant-transmitted-CMV of donor origin. The burned patient also acquires CMV infection but does not generally experience serious complications as regularly as organ recipients, perhaps because burn-related immunosuppression may be less profound than that produced by drugs used to prevent organ rejection. As burn patients begin to receive potent immunosuppressants such as cyclosporine to block rejection of skin allografts, CMV may become a more serious complication of burn care and related blood transfusion and skin allografting. Further studies of skin allograft recipients are needed to determine whether transmission of CMV by skin allograft is associated with symptomatic disease as seen in organ transplantation recipients, or is asymptomatic as generally seen in transfusion-transmitted CMV infections in immunocompetent blood transfusion recipients. It is thus premature to assume that it is beneficial to base selection of skin donors on CMV antibody testing.

EMERGING INFECTIOUS RISKS

Infectious risks of tissue transplantation have often been identified after first being recognised as blood transfusion-transmitted infections. Many real or theoretical risks of tissue transplantation can be considered by looking at the emerging infections that threaten to affect transfusions [92,93]. Most recently West Nile Virus (WNV) infection has swept through the US with nearly 4,000 human cases identified and 254 deaths in 2002 [94]. In addition to being mosquito-borne, WNV has been transmitted through organ transplantation, blood transfusions, transplacental intrauterine spread and percutaneous route from laceration and needlestick [94]. A new corona virus infection recently caused a severe acute respiratory syndrome (SARS) and spread across the continents [95]. Although the susceptibility of these viruses to gamma irradiation or other sterilants is unknown, the routine use of sterilisation may provide some protection from transmission by tissue transplantation.

REDUCING THE RISK THROUGH DONOR SELECTION

To minimise the risk of transmitting viral infectious disease, several important approaches are taken by transplanting surgeons and tissue banks. An initial approach by the surgeon is to judiciously use tissue allografts only when needed and from accredited organisations, use sterilised allografts whenever possible and consider use of autografts and alternative non-human graft material. However, the most important steps are exercised by the tissue bank in excluding those prospective donors who have known or suspected viral infections or are suspected by their behaviours to be at risk for HIV and hepatitis (Table 5). Tissue transplantation is generally considered a non-urgent surgical procedure, permitting a careful tissue donor selection process. Tissue donor selection by tissue banks has evolved to include a direct interview with the donor's next-of-kin concerning the donor's medical history and risk behaviours for HIV and hepatitis along with blood infectious disease testing, a physical examination and the results of an autopsy examination, if performed (Table 6). These donor selection steps are essential activities that result in a low risk of transmitting viruses. In addition, many tissue allografts can undergo further processing and exposure to disinfectants or sterilants, all of which further reduce the hazard of disease transmission [65]. Although there have been no carefully controlled studies of allograft recipients to determine the incidence of disease transmission, there is good reason to believe that established donor screening procedures, infectious disease testing and processing and sterilisation effectiveness to reduce or eliminate viruses, results in a very low risk of transmitting disease.

Table 6. Cadaver tissue donor selection steps to prevent virus transmission.

Voluntary donation without monetary inducement

Health History Review:

- Review of medical records
- Interview of next of kin
- Exclusion of those with infection, no HIV, hepatitis risk behaviour

Blood Tests:

- Hepatitis B surface antigen
- Antibody to HIV-1, HIV-2, HCV
- Antibody to HTLV-I, HTLV-II, syphilis[†]
- Antibody to HBV Core Antigen (for living donors only) [††]
- Nucleus acid test (future)

Physical Examination:

- Unexplained jaundice
- Evidence of injectable drug use
- External signs of infection, including HIV

Autopsy Examination (if performed):

- Exclude those with infection

Maternal HIV testing and risk factor exclusion if donor < 18 months old

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{[†] AATB, EBAA, UNOS & American Red Cross requirements; [††] AATB Requirement}

Donor Selection

One important contribution to recipient safety is to seek voluntary, non-remunerated donors. Monetary inducement to the next-of-kin of cadaveric tissue donors is prohibited by professional standards [2] but it is being considered as a means of reducing the severe organ supply shortage in the US [96].

Monetary payment to donors for blood donation increases the risk of disease transmission [97]. Monetary incentives to donate may cause donors or their next of kin to be untruthful about the donor's health history information and to donate when they should not. Published data clearly show a 5-10 times increased incidence of donor-to-recipient post-transfusion hepatitis B virus infection with the use of paid blood donors [97]. In addition, there is an 11-15 times increased prevalence of HCV antibodies and 3-14 times increased prevalence of HIV antibodies in paid blood donors compared to voluntary blood donors [97]. HCV RNA was detected more often in clotting factor concentrates derived from paid donor plasma than volunteer donor plasma [98].

To minimise the risk of transmitting infectious disease, tissue donor eligibility requirements have been set by national professional standards [2-4,72], US Public Health Service guidelines [99,100], and US federal regulations [73-75]. Donor selection is an important first step taken to ensure that the resulting allograft is safe and effective. The cadaver tissue donor selection process includes a donor's medical and social history obtained from the next-of-kin and medical care providers, blood tests, a physical examination performed by tissue bank personnel, and an autopsy, if performed (Table 6). Preliminary donor selection is based on the donor's medical history and circumstances surrounding death. Donors are excluded if elements of the past medical and social history or recent hospitalisation indicate a risk of infection, malignant disease, or inadequate quality of donated organ or tissue. The living donor, the legal consenting next-of-kin or life partner of a cadaveric donor, or both, must be directly interviewed to determine whether HIV or hepatitis risk behaviours are present (Table 5). Persons with HIV and hepatitis risk behaviours are excluded from donation. The tissue bank physician makes the final determination of suitability of a cadaveric tissue donor as required by national professional standards [2,72].

Physical Examination of Donor

The next screening step is a limited physical examination of the tissue donor by procurement staff at the time of cadaveric donation [2,72]. The body is examined for signs of injecting drug use and signs of HIV, hepatitis or other infection or trauma over bodily sites that can affect the quality of donated tissue.

Blood Testing of Donor

Donor blood testing for disease markers plays an important role in reducing the risk of disease transmission. By eliminating prospective donors with infectious disease risk factors prior to blood testing, the risk of a seronegative but infected donor is minimised. Testing for HBsAg, anti-HIV, and anti-HCV is required by federal regulations [73-75] and national professional standard setting organizations [2,4,72]. Other tests required by standard setting organizations are syphilis and anti-HTLV-I/II [2,4,72].

HIV antigen (p24 antigen) testing of the donor is not performed by most tissue or organ banks. Large-scale studies of low risk [101] and high risk [102] blood donor populations demonstrated a lack of utility for HIV antigen screening. These blood donor studies and similar studies on smaller numbers of cadaver bone [103] and cornea [104] donors did not detect HIV infected donors beyond those already detected by testing for HIV antibodies. However, some regions of the world may have a higher prevalence of HIV infection and in these populations HIV antigen testing may be useful, especially if HIV RNA testing is not performed. One tissue bank has reported finding bone donors with negative tests for HIV antibodies but positive tests for HIV antigen. Presumably these HIV antigen tests were not false positive. The bone bank has added the precaution of treating all bone allografts with 2.5 Mrad gamma irradiation [105].

Studies are underway to determine whether testing donors for HIV RNA and HCV RNA by nucleic acid testing is practical. One study of 1424 cadaver bone donors showed that the use of HIV DNA (not HIV RNA) and p24 antigen blood testing did not detect additional HIV infected cadaveric bone donors [103]. All 1424 donors negative for HIV-1 antibodies were also negative for HIV DNA. This is what is expected since the appearance of HIV DNA in a recently infected person's blood does not occur sooner that the onset of detectable antibodies. On the other hand, HIV RNA appears earlier

and has a greater potential benefit. Although HIV RNA testing is more sensitive than antibody assays, it may be premature to apply it routinely to cadaver donor testing due to the low HIV prevalence in the donor population, its uncertain predictive value, its false positive rate, and its false negative rate due to haemoglobin contamination and other potentially interfering substances in cadaveric post-mortem blood samples.

Testing of living blood donors for HIV and HCV RNA has markedly improved the safety of the blood supply even though screening has been done using pools of 16 to 24 samples [58]. Initially, viral nucleic acid testing was not feasible in blood donor screening applications due to lack of automation, time and space restrictions and cost. Recently, however, test systems are being used to test over 13 million blood donations annually in the United States: the Roche Molecular Systems COBAS AMPLISCREEN tests for HCV and HIV and the Gen-Probe/Chiron Pooled Plasma HIV-1/HCV Amplified Assay. Testing is being done on pooled samples using pools of 24 or 16. Testing of pooled samples reduces the number of tests required on a daily basis, the time to perform testing and the cost. It also takes into account the rapid rise of viral RNA in recently infected individuals so that pooling has a minimal impact on the sensitivity of these assays. The increased sensitivity of these systems over previously available PCR tests has also made this possible. Both systems have now been licensed for blood donor screening and efforts are underway to qualify them for organ and tissue donor screening. The same approach is now being tested in trials for HBV and WNV.

Many bone banks test donors for anti-HBc, a test originally introduced for blood donors as a surrogate for detecting non-A, non-B hepatitis carriers. The utility of this test as a surrogate has been diminished since adding specific tests for HCV (HCV antibodies and HCV RNA), the major cause of non-A, non-B hepatitis [78]. Although not required by AATB Standards and in the absence of HBV DNA testing, the use of anti-HBc in donor testing likely had a safety benefit in reducing HBV infections. Several reports have documented the presence of HBV in the sera of HBsAg-negative, anti-HBc-positive blood donors [79,106,107]. These reports also suggest that the addition of HBV DNA testing will increase the sensitivity of HBV detection but may not entirely replace the need to test for anti-HBc or HBsAg. For example, recipient directed lookback procedures have revealed recipients of HBsAg negative, NAT negative, anti HBc-positive blood components to have been infected with HBV [79].

Hemodilution of Donor Blood Sample

Massive blood loss and intravascular volume replacement by transfusion of blood, colloid, and crystalloid solutions can cause hemodilution and result in unreliable donor test results for infectious diseases [108]. In 1987 a case was reported of HIV transmission to multiple recipients of organs derived from an infected donor. Testing of the donor was negative when blood was sampled immediately after receiving blood transfusions amounting to two to three total blood volumes and an additional large volume of crystalloid solution over an eleven-hour period [109]. When a blood sample was obtained 48 hours later was tested for anti-HIV, it was positive due to intravascular replenishment of immunoglobulin from extravascular sites.

In 1993 US federal regulations [73] were first published, with subsequent modification [74,75], requiring quarantine of tissue from adult donors who had blood loss and received greater than two litres of blood and colloids within 48 hours of blood sampling or greater than one litre of crystalloid within a one hour of sampling. The donated tissue was not to be used unless a pre-transfusion sample was available for testing or an algorithm used to evaluate blood and colloid volumes administered in the 48 hours prior to sampling to ensure sufficient plasma dilution to alter test results has not occurred.

AATB Standards also require tissue banks to follow written procedures setting hemodilution limits to prevent use of false negative results when testing post-transfusion blood samples for infectious disease [2]. Acceptability limits must be part of written procedures. Standards of the American Red Cross Tissue Services require that in the case of blood loss and transfusion within 48 hours of death, a pre-infusion sample must be used [72]. A post-infusion sample may be used in patients with major clinical blood loss provided that the tissue bank physician has evaluated whether blood and crystalloid infusions have compensated for blood loss, estimated hemodilution is 50 percent or less of the total blood volume and the tissue bank physician gives written approval. The estimated amount of hemodilution depends upon the type of fluid infused and the amount of time elapsed since infusion.

Quality of Cadaver Donor Blood Samples

The testing of cadaveric tissue donor serum for viral markers may be complicated by false positive tests when sampling is delayed after death [104] or when there is haemolysis [53,104]. False positive results for HBsAg and p24 antigen due to haemolysis may be found depending upon which manufacturer's test kit is used [53]. Sample quality and presence of haemoglobin can also cause false negative results when testing for HIV and HCV by NAT [110,111]. Frozen storage and multiple freeze-thaws do not have a major effect on detectability of antibodies to infectious agents in serum, but they may reduce the reliability of testing for microbial nucleic acids by NAT. Busch *et al.* showed that multiple freeze thaws can reduce the detectability of HCV RNA by NAT [112]. Published studies are too few to draw any firm conclusions, other than a possible deleterious effect of frozen storage and freeze thawing on testing serum for HCV RNA and peripheral blood white cells for HIV DNA by NAT. Despite this, NAT was used to detect and retrospectively diagnose HIV infection using marrow specimens from an organ and tissue donor and frozen sera from organ recipients 5 years after the donation and transplantation [42]. HCV testing by NAT was also useful in confirming HCV infection in one cadaveric donor [38] and was essential in another [39]. Although NAT for hepatitis and HIV will be very useful, the tests are under development for organ and tissue donor testing.

Autopsy

Autopsies of donors are not generally required but for those tissues that can be stored, a final donor suitability determination is not made by the tissue bank physician until the results of the autopsy, if performed, have been reviewed [2,72]. Autopsy findings may not be reliable in detecting viral infections but results have disqualified donors with undiagnosed malignancy, widespread granulomas, abscess, and pneumonia.

Living Bone Donor Selection

Femoral heads can be donated by persons undergoing hip arthroplasty. Donor medical history screening and testing requirements are similar to those for cadaver bone donors, except that the donor medical history interview can be made directly and a retest for HIV and HCV antibodies is required 180 days following donation [2,72,99,100]. The retest aims to identify recently infected donors who were seronegative for HIV and HCV antibodies at the time of donation. This 180 day retest is required for semen and bone donors but not for living donors of blood, marrow, amnion, umbilical veins, or

foetal tissue. Retesting the low risk voluntary living bone donors has not detected any additional infections despite thousands of donations and retests [61], whereas it may have utility in the higher risk population of paid semen donors. Most tissue banks have ceased collecting surgical femoral heads, partly due to lower quality, but also due to the difficulties of acquiring the 180-day sample for retesting. Testing of samples for HCV and HIV RNA from living donors at the time of donation would enhance safety and could eliminate the need for a 180-day retest.

REDUCING THE RISK DURING CADAVERIC TISSUE PROCESSING

Tissue allografts can be contaminated by bacteria or fungus during processing from environment surfaces and air, from personnel, from contaminated reagents, surgical instruments, supplies and processing equipment. In cases of HIV and HCV transmission by tissue allografts, the origin of the virus has been from infection of the donor. Electrolyte solutions purchased commercially [113] or deionised water prepared by the tissue bank [114] can be contaminated by bacteria and contaminate the tissue allograft when used for processing. In rare cases, there has been HBV contamination acquired during liquid nitrogen storage [115]. This has led to the widespread practice of using the vapour phase rather than submersion in the liquid phase. Viral contamination during tissue processing has not been reported.

The bone graft disinfection and sterilisation step most often used by bone banks in the United States is gamma irradiation at doses of 15 to 30 kGy [116]. For several types of tissue, processing steps involve elimination of blood cells and exposure to disinfectants such as alcohol and peroxide, both of which have viral inactivation properties. The use of sterilants such as gamma irradiation, ethylene oxide and heat, are more reliable for eradicating viable viruses.

SUMMARY

Transplantation of tissues has resulted in transmitting viral diseases from donor to recipient. When the first truly effective immunosuppressant, cyclosporin, became available in 1981, organ transplantation flourished. The numbers of organs transplanted each year grew rapidly leading to implementation of effective programs to develop public support for organ donation. As organ donation grew, so did tissue donation. With the rapid growth of transplantation, early cases of transplant transmitted viral diseases arose from infections of the donor but could have been prevented if tests were available. The use of new and more sensitive donor viral detection tests has reduced the risk of recipient infections.

Early cases of transmitted viral infection involved donors who had HIV and hepatitis risk behaviours. Exclusion of these as prospective donors has played an important role in reducing the risk of transmission. More recently, several cases have been reported where the infecting organism is bacterial and fungal instead of viral and the microbes did not arise from the donor but was a contaminant acquired during the procurement, processing or storage of the tissue. Newer national professional standards and US FDA regulations are addressing this area.

To prevent, or at least minimise, the risk of transmission of infectious disease, several approaches are important. Surgeons should use allografts only when needed and obtain them from accredited tissue banks. They should consider use of autografts, alternative nonhuman graft material, or processed and sterilised tissue allografts whenever possible tissue banks reduce the risk for rejecting those donors suspected to

be at risk for HIV, hepatitis, or other viral or bacterial infections, performing a donor physical examination, reviewing autopsy reports, and the surgical removal of donated tissue using aseptic technique. Lastly, sterilisation of certain tissues can be very effective, but it is not universally applicable, because some viruses are relatively resistant to sterilants and because the clinical effectiveness of many tissues (such as skin, cornea, valves, veins) can be altered by sterilisation procedures [117]. Application of all these steps will assure a very low risk of transmitting disease from the donor to the recipient.

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