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Transmission of Hepatitis C Virus to Several Organ and Tissue Recipients from an Antibody-Negative Donor

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Background: **Although hepatitis C virus (HCV) transmission through tissue transplantation has been rarely reported, a donor with undetected viremia may infect several recipients. A patient developed acute hepatitis C shortly after tissue transplantation. Ninety-one tissues or organs had been recovered from the donor.**

Objective: **To determine whether the donor was the source of infection and the extent of transmission to other organ and tissue recipients.**

Design: **Descriptive epidemiologic study; serum testing for HCV infection.**

Setting: **Recipients were located in 16 states and 2 other countries.**

Participants: **Donor and graft recipients.**

Measurements: **Hepatitis C virus infection was defined as the presence of anti-HCV or HCV RNA. The authors determined the genetic relatedness of viral isolates from the donor and recipients by genotype comparison and quasi-species analysis.**

Results: **The donor was anti-HCV–negative but was HCV RNA–positive (genotype 1a). Forty persons received transplants during 22 months. Five persons were HCV-infected before transplantation or had a genotype other than 1a, and 5 persons had no post-transplantation serum specimens available. Of the remaining 30 recipients, HCV infection occurred in 8 recipients: 3 of 3 organ recipients, 1 of 2 saphenous vein recipients, 1 of 3 tendon recipients, and 3 of 3 tendon with bone recipients. These 8 recipients had viral isolates genetically related to those of the donor. No cases occurred in recipients of skin ($n = 2$), cornea ($n = 1$), or irradiated bone ($n = 16$).**

Limitations: **Post-transplantation serum specimens were unavailable for 5 recipients.**

Conclusions: **An anti-HCV–negative donor was the source of HCV infection for 8 recipients of organs or tissues. Although HCV transmission from anti-HCV–negative donors is probably uncommon, changes in donor screening to include routine testing for HCV RNA merit further consideration to improve the safety of transplantation.**

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Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease in the United States, and an estimated 2.7 million persons are chronically infected (1). Transmission has occurred principally through percutaneous blood exposures, such as injection drug use and receipt of infected blood products before 1990, before routine screening for HCV.

Hepatitis C virus transmission through organ transplantation has been well described (2–9). In contrast, HCV transmission through tissue transplantation has been described rarely and only through nonirradiated, frozen, or cryopreserved grafts of bone or tendon with bone (10–12). No transmission through tissue transplantation has been reported from a donor found negative for antibody to HCV (anti-HCV) with a second- or third-generation enzyme immunoassay.

Musculoskeletal graft procedures are increasingly common. In 2003, 1.2 million bone graft procedures were performed in the United States, compared with 350 000 procedures in 1990 (13). Because 1 donor may be the source of several organs and approximately 100 tissues, 1 infected donor can transmit infection to many recipients across the nation and world. To minimize the risk for HCV transmission, organ and tissue donors are screened for risk factors for HCV infection and tested for anti-HCV, but nucleic acid testing to detect HCV RNA is not required by the U.S. Food and Drug Administration (FDA) (14–17).

In June 2002, a physician reported to Oregon public health officials a case of acute, symptomatic hepatitis C in a patient who had received a patellar tendon with bone allograft approximately 6 weeks before onset of symptoms. The tissue donor had died in October 2000, and his premortem serum had been anti-HCV–negative with a second-generation enzyme immunoassay. Ninety-one tissues or organs had been recovered from this donor. We investigated to determine whether the donor was the source of HCV infection and the extent of transmission to other organ and tissue recipients.

METHODS

Epidemiologic Investigation

We reviewed the donor's medical charts and tissue bank records for evidence of liver disease and risk factors

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for HCV infection. We reviewed questionnaires administered by the organ procurement agency to the donor's next of kin and by the tissue bank to the primary care physician to determine risk factors for HCV infection. A pathologist reviewed archived slides of a liver biopsy performed 18 hours before organ recovery.

Through the tissue banks, the organ procurement agency, and the eye bank, we acquired a list of grafts and preparation methods, as well as recipient names or contact information of the transplanting facilities. We arranged for testing of recipient serum for HCV infection. We interviewed HCV-infected recipients or reviewed available laboratory records and medical charts for details on the diagnosis of hepatitis C. We defined a case as HCV infection in a recipient who was not known to have been infected before transplantation, with viral isolates genetically related to those of the donor, as determined by genotype and quasi-species analysis.

Laboratory Testing

We obtained premortem donor serum that was collected before the receipt of any blood or blood products and stored frozen by the transplant bank. We tested donor serum for anti-HCV by using a third-generation enzyme immunoassay (ORTHO HCV Version 3.0 ELISA [enzyme-linked immunosorbent assay], Ortho-Clinical Diagnostics, Raritan, New Jersey). We also tested and quantified donor serum for HCV RNA by using AMPLICOR HCV Test, version 2.0 (lower limit of detection, 50 IU/mL), and AMPLICOR HCV MONITOR Test, version 2.0 (Roche Molecular Systems, Branchburg, New Jersey), respectively.

We arranged for testing of recipients' post-transplantation serum specimens and, when available, stored pretransplantation specimens. Recipient serum specimens were tested for anti-HCV by using either a second-generation (Abbott HCV EIA 2.0, Abbott Laboratories, Abbott Park, Illinois) or third-generation enzyme immunoassay (ORTHO HCV Version 3.0 ELISA). We verified serologic test results showing anti-HCV with a recombinant immunoblot assay (RIBA, Chiron Corp., Emeryville, California). We tested all serum specimens for HCV RNA (AMPLICOR HCV Test, version 2.0). We considered a recipient to have HCV if we detected either anti-HCV or HCV RNA.

We determined HCV genotypes from a 300-nucleotide NS5B coding region by using previously described methods and compared them with published sequences of known genotypes (1, 18–21). Any recipient sharing the same genotype and 95% or more of NS5B sequence nucleotides with the donor underwent genetic testing with quasi-species analysis. Quasi-species, or closely related populations of viruses that share a common origin, occur within HCV-infected individuals because of errors during HCV replication over time. We determined the distribution of quasi-species by sequencing hypervariable region 1 from different viral isolates (amplicons) amplified from each individual using methods described previously (18). For comparison, we also performed quasi-species analysis from randomly selected HCV-infected individuals (also sharing ≥95% of NS5B sequence nucleotides with the donor) from the Third National Health and Nutrition Examination Survey (NHANES III) (1), a representative sample of the noninstitutionalized civilian population of the United States.

We conducted pairwise analysis (PileUp and Pretty, Wisconsin Package, Genetics Computer Group, Madison, Wisconsin), calculated the distribution of nucleotide distances (Evolutionary Distances, Wisconsin Package), and generated an unrooted phylogenetic tree (DNADIST and NEIGHBOR programs with PHYLIP [Phylogeny Inference Package], version 3.5, Department of Genome Sciences, University of Washington, Seattle, Washington). A phylogenetic tree is a graphical way to depict the evolutionary relationships (variation) among sequences of interest. The lengths of the branches are proportional to the nucleotide distance between sequences. We performed a bootstrap analysis, generating 1000 pseudosamples and pseudotrees by resampling the dataset to evaluate the reliability of the phylogenetic tree (22).

Context

Transmission of hepatitis C virus (HCV) infection as a result of tissue transplantation has not been previously reported from donors testing negative with a second- or third-generation enzyme immunoassay.

Contribution

Stored tissues (3 organs and 27 tissues) from an anti-HCV–negative donor (by second-generation immunoassay) were transplanted into 30 recipients known to have been HCV negative before transplantation. Five tissue recipients and all 3 organ recipients were infected with HCV.

Implications

In view of the large number of potential recipients from a single tissue donor, HCV RNA testing of donors may improve the safety of organ and tissue transplantation.

—The Editors

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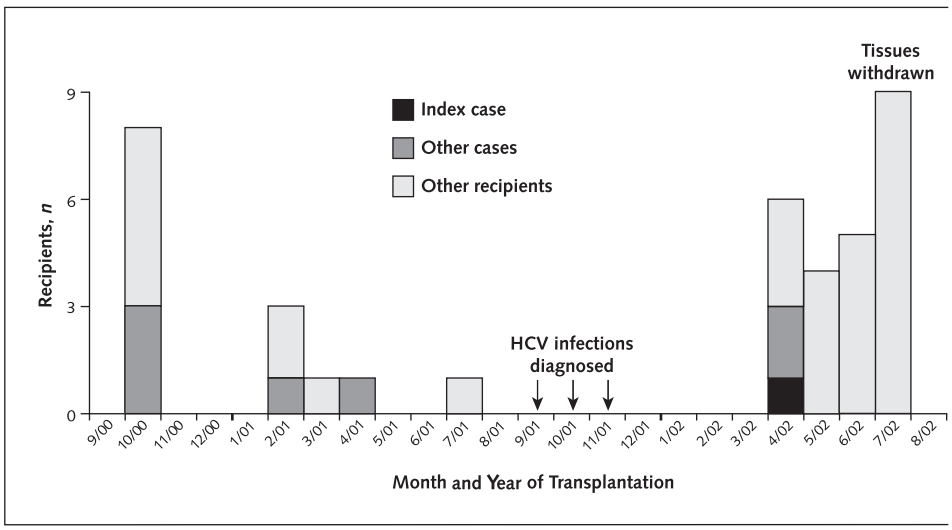
The funding source had no role in the design, conduct, or reporting of the study or in the decision to submit the manuscript for publication.

RESULTS

Investigation of Donor

The donor was a man in his 40s with a history of hypertension and heavy alcohol use who had died of an intracranial hemorrhage in October 2000. At the time of death, liver aminotransferase levels were normal and phys-

Figure 1. **Transplantation of grafts from a donor with hepatitis C virus (*HCV*) infection, by month of transplantation and case status (*n* = 38), United States, 2000–2002.**



Dates of transplantation for 2 recipients were unknown. Hepatitis C virus infection was diagnosed in 3 recipients in September, October, and November 2001. These recipients had undergone transplantation in February 2001, October 2000, and April 2001, respectively.

ical examination showed no signs of liver disease or injection drug use. A liver biopsy performed before organ recovery showed mildly active steatohepatitis without significant fibrosis.

The medical and social history solicited from the donor's next of kin revealed no history of injection drug use or blood transfusions. The questionnaire completed by the donor's primary care physician indicated that he was unaware of a history of hepatitis or any reason why the patient should be excluded from donation.

In July 2002, stored donor premortem serum was tested and confirmed to be anti-HCV–negative. However, HCV RNA was detected, with a viral load of 3.6×10^6 IU/mL (genotype 1a).

Preparation of Grafts

Ninety-one grafts were produced from the donor (7 organs, 2 corneas, and 82 noncorneal tissues). Organs had been flushed with and stored in an electrolyte preservation solution (Viaspan, Barr Laboratories, Pomona, New York) containing insulin, dexamethasone, and penicillin. In preparation for cornea excision, the whole eye had been soaked in a povidone-iodine solution and rinsed in saline. After excision, the corneas had been placed in a nutrient medium with antibiotics.

The skin, saphenous veins, and tibialis tendons had undergone an antimicrobial wash and cryopreservation but no irradiation. The patellar and Achilles tendon with bone grafts had been lavaged with sterile water and then soaked in Allowash Solution (LifeNet, Virginia Beach, Virginia), isopropyl alcohol, antibiotics, and sterile water. They had been fresh-frozen but not irradiated. Bone grafts had been lavaged with sterile water; put through ultrasonication and centrifugation; and subjected to Allowash Solution, peroxides, and antibiotics, followed by isopropyl alcohol and sterile water soaks. The bone grafts had then been lyophilized, with a residual moisture content of 2.20% and 2.27% according to 2 quality-control samples, followed by 16.4 kGy to 19.7 kGy of γ-irradiation through a cobalt-60 source at room temperature.

Investigation of Recipients

Of the 91 grafts produced, 44 had been transplanted into 40 recipients in 16 states and 2 other countries. Six persons received organs, 34 persons received tissues (including 2 corneas), and 1 person received 5 skin grafts. Grafts had been transplanted over a 22-month period (**Figure 1**). The index patient had received her transplant in April 2002 and received a diagnosis of acute HCV infection in June 2002. In July 2002, the tissue bank voluntarily withdrew 44 remaining tissues from distribution after notification of possible HCV transmission; 2 tissues and 1 organ were previously discarded.

Of the 40 recipients, 4 were reported to have been HCV-infected before transplantation (documentation unavailable for 2 recipients), 1 recipient was found to be HCV-infected with genotype 3a post-transplantation, and 5 recipients had no post-transplantation serum specimens available to test (including 1 recipient whose name was not retained by the transplanting facility) (**Table**).

Of the remaining 30 recipients, including 3 organ recipients and 27 tissue recipients, 8 (all 3 organ recipients and 5 tissue recipients) were found to be HCV-infected (**Table**). The remainder were HCV-negative when tested at least 6 months after transplantation.

The donor and these 8 HCV-infected recipients were infected with genotype 1a, with identical NS5B region sequences. Serum specimens were available from 3 other re-

cipients who were known to have been infected before transplantation. One of the 3 recipients shared 95% or more of NS5B sequence nucleotides with the donor and therefore underwent quasi-species analysis. We chose 3 NHANES III participants for quasi-species analysis. That analysis showed that the 16 amplicons isolated from the donor had an identical hypervariable region sequence. The similarity (percentage of nucleotides shared in the hypervariable region 1) between the donor sequence and 5 to 31 amplicons sequenced from each of the 8 recipients ranged from 96% to 100%. The sequences from the donor and the 8 recipients clustered in 1 group when compared in an unrooted phylogenetic tree (**Figure 2**). (For this cluster, the bootstrap value was 95%, meaning that we observed the clustering of sequences presented in **Figure 2** in 95% of the 1000 pseudotrees generated.) In contrast, sequences from the NHANES III participants (30 to 40 amplicons each) and 1 recipient infected before transplantation (27 amplicons) were located on distinct branches. Similarities to the donor sequence ranged from 68% to 74% in the NHANES III group and from 59% to 69% in the amplicons from the 1 recipient infected before transplantation.

Among the 8 case-patients, median age at transplantation was 54 years; 4 patients were women. The 3 HCV-infected organ recipients had been HCV-negative, as determined by testing of frozen serum specimens collected up to 3 days before transplantation. We tested serum specimens from the organ recipients collected 4 days, 6 weeks, or 21 months after transplantation and detected HCV RNA in all 3 recipients. However, each recipient tested anti-HCV–negative. The organ recipient whose serum was tested on day 4 after transplantation was tested by her own physicians 1 year after transplantation with the same anti-HCV–negative result. Two infected patients, both lung recipients, died. One died of causes apparently unrelated to liver disease, while the other had presented several months after transplantation with abdominal pain and ascites, which led to a diagnosis of HCV infection. She died with end-stage liver disease 14 months after transplantation.

The 5 cases among tissue recipients included 1 saphenous vein recipient, 1 tibialis tendon recipient, and 3 tendon with bone recipients (including the index case). None had pretransplantation serum available for testing. Two of these patients, in addition to the lung recipient, had received a diagnosis of HCV infection several months before the index patient's transplantation (**Figure 1**); however, the infection was not recognized as allograft-associated at that time. No cases occurred in recipients of cornea ($n = 1$), skin ($n = 2$), or irradiated bone ($n = 16$).

DISCUSSION

An antibody-negative, HCV-infected donor was the source of organs and tissues for 40 recipients, 8 of whom developed infection as a result. Onset time of acute hepatitis C in the index patient was consistent with transmission at the time of transplantation. We documented new infection in 3 organ recipients who had tested negative before transplantation. Viral isolates of the donor and case-patients were closely genetically related, by both genotype and quasi-species analysis.

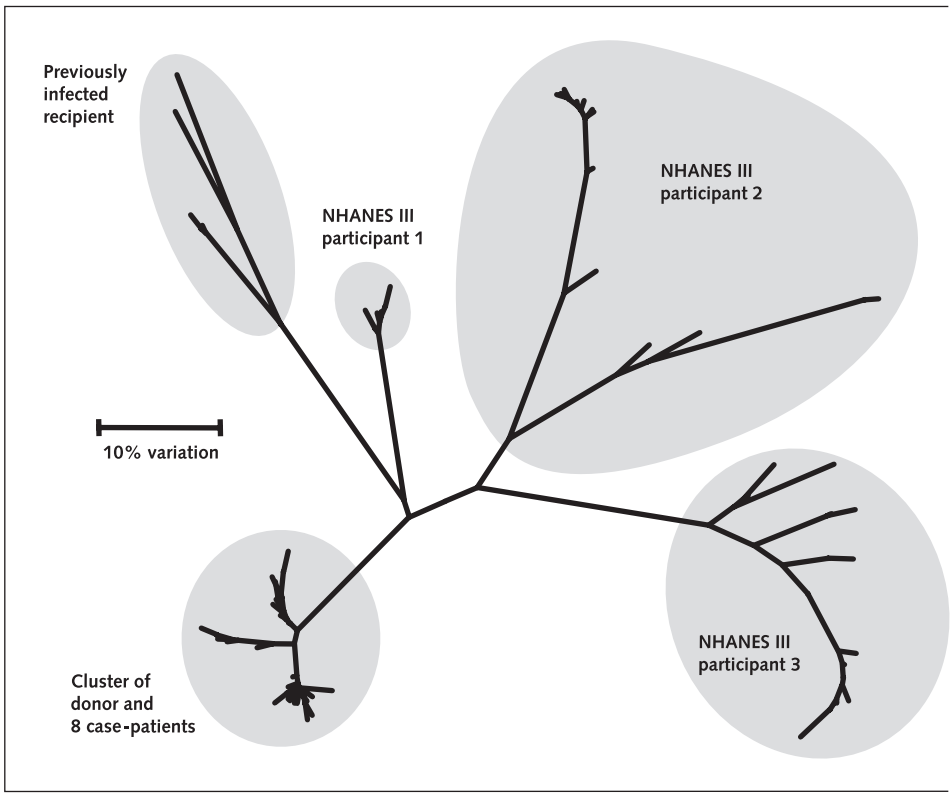
Donor screening is the primary way to prevent transmission of viral infections from organs and tissues. Required tissue donor screening includes assessment of risk

Table. **Classification of Graft Recipients from a Hepatitis C Virus–Infected Donor, United States, 2000–2002***

Graft Type	Manner of Processing	Recipients, *n*	Classification				Proportion Infected, *n*/*n*§
			Unassociated HCV Infection, *n*†	Unable To Test Post-Transplantation, *n*	HCV-Negative, *n*	Cases, *n*‡	
Organ	Fresh	6	0	3‖	0	3	3/3
Cornea	Fresh	2	1	0	1¶	0	0/1
Skin	Cryopreserved	2	0	0	2	0	0/2
Saphenous vein	Cryopreserved	2	0	0	1	1	1/2
Tibialis tendon	Cryopreserved	4	1	0	2	1	1/3
Tendon–bone	Fresh frozen, Allowash**	4	0	1††	0	3	3/3
Bone	Lyophilized, Allowash**, irradiated	20	3	1‡‡	16	0	0/16
Total		40	5	5	22	8	8/30

* HCV = hepatitis C virus.
† These recipients were HCV-infected but did not meet the case definition. Four were reported to have been infected before transplantation (documentation unavailable for 2 recipients), and another was infected with HCV genotype 3a.
‡ A case was defined as HCV infection in a recipient who was not known to have been infected before transplantation, with viral isolates genetically related to those of the donor.
§ Defined as cases divided by the total number of cases and HCV-negative recipients.
‖ Post-transplantation serum unavailable; recipients deceased. One of these recipients, a kidney recipient, had been anti-HCV–negative 7 mo after transplantation, but no HCV RNA testing had been performed.
¶ The recipient had negative anti-HCV (Abbott HCV EIA 2.0, Abbott Laboratories, Abbott Park, Illinois) and negative qualitative HCV RNA test results 2 years after transplantation, but information on the specific HCV RNA assay used is unavailable.
** LifeNet, Virginia Beach, Virginia.
†† Recipient located in another country with limited information available.
‡‡ Recipient's name was not retained by the transplanting hospital.

Figure 2. **Unrooted phylogenetic tree of hypervariable region sequences of graft donor, graft recipients, and selected Third National Health and Nutrition Examination Survey (*NHANES III*) participants, United States, 2000–2002.**



Each branch represents a different viral sequence, and small distances between branches suggest genetic relatedness. Sequences belonging to each NHANES III participant and a recipient known to have been infected before transplantation are marked by separate shaded regions. The size of each shaded region represents the genetic diversity of quasi-species from that specimen or group of specimens. Isolates belonging to the donor and 8 case-patients are clustered together.

factors for and clinical evidence of hepatitis C and testing of serum (14). Proxy surveys with the donor's next of kin and physician did not uncover risk factors. Organ and tissue donors are required to be tested for anti-HCV, and the FDA is considering but not yet requiring HCV nucleic acid testing (14, 16).

In our investigation, the donor was probably in the 8- to 10-week "window period" of infection before the development of detectable anti-HCV (23). Barriers to nucleic acid testing in organ and tissue donors exist. Because organs must be transplanted quickly, nucleic acid testing may be impractical given the 1 to 2 days that it requires. By contrast, tissues can often be stored for months to years before use, allowing ample time. However, tissues are often recovered from cadaveric donors, and the FDA only recently approved a nucleic acid test for use in postmortem serum specimens (24). The FDA may recommend such a test in the future, pending further validation of the test's accuracy in cadaveric specimens (14, 16).

While nucleic acid testing might detect cases in the window period, donors in this period are probably rare. A recent analysis estimated the probability of undetected viremia with HCV in antibody-negative tissue donors to be 1 in 42 000 donors (25). The actual rate of transmission is unknown, since most tissues undergo processing to reduce the risk. The authors determined that the probability of viremia with HCV would be reduced to 1 in 421 000 donors if nucleic acid testing were performed on individual donors and that the cost of eliminating 1 HCV-infected donor would be $2.3 million, spread over approximately 1 million tissue products per year. Compared with tissue donors, the incidence of hepatitis C viremia among antibody-negative blood donors is lower (26).

Our investigation suggests that not all tissues carry the same risk for transmission. Previously, only nonirradiated bones and tendons with bone have been reported to transmit HCV infection (10–12). Similarly, we report transmission of HCV to recipients of nonirradiated tendons with bone. We believe that this is the first documented transmission through saphenous veins and tendons without bone.

In contrast, skin, corneas, and γ-irradiated bone did not transmit HCV. Absence of HCV transmission through corneas (10, 12) and γ-irradiated bone and soft tissue (12) has been noted previously. Factors limiting transmission from skin and corneas are unknown but might include tissue vascularity, graft size, or virus concentration. While irradiation might be virucidal, at high doses it can impair

tissue biomechanical integrity (27, 28). More data are needed on the optimal use of alternate tissue sterilization techniques, including low-temperature chemical sterilization processes (29).

All 3 organ recipients tested were infected through transplantation. Perhaps because of their immunocompromised state, they did not develop HCV antibody (although 1 recipient may have been tested too early to detect anti-HCV, only 6 weeks after transplantation). Therefore, in an organ recipient, anti-HCV testing alone seems to be insufficient to exclude the possibility of infection.

Detection of allograft-associated HCV infections is difficult. This outbreak was not detected until nearly 2 years after the donor's death. Recipients are often geographically distant from each other, and HCV infections are usually asymptomatic and are not notifiable in every state. Several months before the index patient presented with hepatitis C, 3 recipients had received a diagnosis of HCV infection that was not recognized as organ transplantation– or tissue graft–associated. Earlier investigation might have prevented further cases. When a new case of hepatitis C is diagnosed in an allograft recipient, the health care provider should notify public health authorities, so that tissues from infected donors can be removed from distribution and recipients can be evaluated for infection.

Our investigation was limited by incomplete information for some recipients. One recipient could not be identified by the transplanting facility. Facilities receiving grafts should keep accurate records to facilitate epidemiologic investigation, tissue recall, and patient notification. The FDA recently finalized 3 comprehensive new rules for a broad range of human cells, tissues, and cellular- and tissue-based products in an effort to improve their safety and prevent transmission of communicable disease (14, 17, 30).

An antibody-negative, HCV-infected tissue and organ donor is probably rare but may infect many recipients. Tissues vary in their ability to transmit HCV infection, and some carry a low risk. Enhanced donor screening including HCV nucleic acid testing, as well as improved tissue processing techniques, record keeping, and reporting of adverse events, may further improve the safety of tissue and organ transplantation.

From the Centers for Disease Control and Prevention, Atlanta, Georgia; Oregon Department of Human Services, Portland, Oregon; and Community Blood Center, Community Tissue Services, and Wright State University School of Medicine, Dayton, Ohio.

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