American Journal of Transplantation 2015; 15: 1827–1835
Wiley Periodicals Inc.

doi: 10.1111/ajt.13283

Transmission of Hepatitis C Virus From Organ Donors Despite Nucleic Acid Test Screening

A. Suryaprasad[1,*], S. V. Basavaraju[2], S. N. Hocevar[2], N. Theodoropoulos[3], R. A. Zuckerman[4], T. Hayden[1], J. C. Forbi[1], D. Pegues[5], M. Levine[5], S. I. Martin[3], M. J. Kuehnert[2], E. A. Blumberg[5] on behalf of the Organ Transplantation Hepatitis C Investigation Team[†]

[1]Division of Viral Hepatitis, US Centers for Disease Control and Prevention, Atlanta, GA
[2]Division of Healthcare Quality and Promotion, Office of Blood, Organ and Other Tissue Safety, Centers for Disease Control and Prevention, Atlanta, GA
[3]Department of Internal Medicine, Division of Infectious Diseases, The Ohio State University, Columbus, OH
[4]Section of Infectious Disease and International Health, Dartmouth-Hitchcock Medical Center, Lebanon, NH
[5]Perelman School of Medicine at the University of Pennsylvania Health System, Philadelphia, PA
[*]Corresponding author: Anil Suryaprasad, asuryaprasad@cdc.gov, anil.suryaprasad@mayo.edu, anilsury1@gmail.com
Correction made after online publication May 5, 2015: Affiliations have been updated.

Nucleic acid testing (NAT) for hepatitis C virus (HCV) is recommended for screening of organ donors, yet not all donor infections may be detected. We describe three US clusters of HCV transmission from donors at increased risk for HCV infection. Donor's and recipients' medical records were reviewed. Newly infected recipients were interviewed. Donor-derived HCV infection was considered when infection was newly detected after transplantation in recipients of organs from increased risk donors. Stored donor sera and tissue samples were tested for HCV RNA with high-sensitivity quantitative PCR. Posttransplant and pretransplant recipient sera were tested for HCV RNA. Quasispecies analysis of hypervariable region-1 was used to establish genetic relatedness of recipient HCV variants. Each donor had evidence of injection drug use preceding death. Of 12 recipients, 8 were HCV-infected—6 were newly diagnosed posttransplant. HCV RNA was retrospectively detected in stored samples from donor immunologic tissue collected at organ procurement. Phylogenetic analysis showed two clusters of closely related HCV variants from recipients. These investigations identified the first known HCV transmissions from increased risk organ donors with negative NAT screening, indicating very recent donor infection. Recipient informed consent and posttransplant screening for blood-borne pathogens are essential when considering increased risk donors.

Abbreviations: 5′-UTR, 5′ untranslated region; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CDC, US Centers for Disease Control and Prevention; DTAC, Disease Transmission Advisory Committee; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HVR-1, hypervariable region-1; IDU, injection drug use; IU, international units; mL, milliliter; NAT, nucleic acid testing; NS5B, nonstructural protein 5B; OPTN, Organ Procurement and Transplantation Network; PHS, US Public Health Service; UNOS, United Network for Organ Sharing

Received 10 November 2014, revised 23 December 2014 and accepted for publication 08 January 2015

Introduction

The risk of infection transmission via solid organ or tissue transplantation has been recognized for decades, and improving the safety for recipients remains a worldwide concern (1). Nearly 80 000 US patients are on active waiting lists for organ transplantation, while over 6000 die annually while waiting (2,3). As a result, there has been greater use of more extended criteria donors (4). Donors with behaviors associated with an increased risk for recent infection with human immunodeficiency virus (HIV),

[†]Organ Transplantation Hepatitis C Investigation Team: Pennsylvania Department of Health: Lauren Torso, MPH; Lifeline of Ohio: Dorrie Dils, RN, BSN, MHA, CPTC; New England Organ Bank: Larry Sussman, CTBS, CQA; Division of Viral Hepatitis, US Centers for Disease Control and Prevention: Guoliang Xia, PhD, Sumathi Ramachandran, PhD, Yury Khudyakov, PhD, Saleem Kamili, PhD, Jan Drobeniuc, MD, PhD; The Ohio State University: Elmahdi Elkhammas, MD (Division of Transplant Surgery), Amy Pope-Harman, MD (Division of Pulmonary Medicine), Bryan Whitson, MD, PhD (Division of Cardiac Surgery), Nicholas Dipaola, MD (Histocompatibility Laboratory); Johns Hopkins Medicine: Pali Shah, MD; University of Pennsylvania Transplant Center: Lee Goldberg, MD, MPH, Melissa Bleicher, MD, Stacey Doll, MPA, Peter Abt, MD, Karen Krok, MD; Dartmouth-Hitchcock Medical Center: Michael C. Chobanian, MD (Department of Surgery), David A. Axelrod, MD (Department of Surgery), Nancy Dunbar, MD (Departments of Medicine and Pathology); Yale School of Medicine: David B. Banach MD, MPH (Section of Infectious Diseases).

hepatitis C virus (HCV) and hepatitis B virus (HBV), known as increased risk donors, are increasingly accepted for transplant, especially as they tend to be younger and in otherwise good health (5). Although they are deemed to be at increased risk for possible unexpected viral pathogen transmission, the actual risk for transmission through organ transplantation is rare (6–9). However, when transmissions occur, they often result in serious illness and death in organ recipients (10–14).

In 2013, the US Public Health Service (PHS) published guidelines with revised criteria for recognizing donors at increased risk for transmission of HIV, HBV and/or HCV (15). Among 12 criteria that determine increased risk is nonmedical injection drug use (IDU) (15). Due to the long window period of HCV serologic detection (7–10 weeks) (15–18), which has resulted in HCV transmissions despite negative serologic screening (7–9), and the unreliability of donor next-of-kin history for HCV risk factors, the PHS guidelines now recommend HCV nucleic acid testing (NAT) as screening of all deceased donors prior to organ procurement (15). Consistent with PHS guideline recommendations, Organ Procurement and Transplantation Network/United Network for Organ Sharing (OPTN/UNOS) policy mandates informed consent of recipients who receive increased risk donor organs and recommends posttransplant follow-up for all recipients receiving organs from increased risk donors (15).

Despite these measures, transmission may not be fully averted. NAT has a 7–10-day period preceding testing, virologically defined as an eclipse phase, when HCV infection might go undetected (16). To date, transmission of HCV through organ transplantation during this period of NAT has not been described in the literature. Risk behaviors, including IDU, during this period might result in new acute donor infection, which would be undetected by NAT. Potential donor-derived disease transmission events are reported to the OPTN per policy and reviewed by the *ad hoc* OPTN Disease Transmission Advisory Committee (DTAC) to determine the likelihood of disease transmission (19,20). Through an agreement with the OPTN/DTAC, the US Centers for Disease Control and Prevention (CDC) is represented on DTAC and leads investigations of select cases of public health importance. We describe three clusters of solid organ transplant–transmitted HCV infections reported to CDC, which occurred despite negative HCV NAT and serologic testing of the donors prior to organ procurement. Implications for donor selection and safe donor use are discussed.

Methods

Epidemiologic investigation

Cases were defined as solid organ recipients with either nonreactive anti-HCV antibody or undetectable HCV NAT in the 6 months prior to transplant, but with detectable HCV RNA within 12 weeks of transplantation. Medical records were reviewed for each case and respective organ donors. Recipient medical record review included ascertaining any exposures other than organ donation that may have resulted in HCV infection including high-risk behaviors (e.g. IDU, intranasal drug users) or healthcare exposures such as receipt of injectable medications and percutaneous procedures or surgeries in the 2 weeks preceding transplantation until the date of HCV detection after transplantation. Similarly, donor medical records were reviewed for evidence of increased risk behavior and any healthcare exposures in the 2 weeks preceding death.

A standardized acute case report questionnaire to identify routinely reported HCV risk factors was administered to all infected recipients (21). Furthermore, infection control practices within donor and recipient facilities were reviewed using standardized CDC guidance to identify opportunities for blood-borne pathogen transmission (22). Narcotics safeguards and administration practices were evaluated by hospital infection control practitioners to determine whether HCV transmission occurred secondary to diversion. Employee logs and surgical records were reviewed to identify any common healthcare personnel in surgical procedures of newly infected recipients and any documented needle stick injuries. A trace back of blood products, which involved voluntary re-testing of identifiable donors, was attempted for blood components transfused to either the donor or recipients in the 2 weeks before transplant and to recipients in the weeks following transplant surgery before HCV diagnosis.

Laboratory methods

Laboratory testing was conducted at CDC. Stored serum from each organ donor was re-tested with the high-sensitivity quantitative COBAS AmpliPrep/COBAS Taqman HCV v2.0 PCR assay (Roche Molecular Systems, Branchburg, NJ). Serum specimens (posttransplant and if available stored pretransplant) were obtained from recipients and tested with the same COBAS platform for evidence of HCV infection. Nucleic acid was extracted from donor splenocytes or lymphocytes, which are archived from each organ donor at organ recovery for histocompatibility typing. The extracted nucleic acid was subjected to amplification and testing for detection of HCV RNA with an in-house Taqman assay (23).

Seven confirmed genotypes and 67 subtypes of HCV exist (24). The most heterogeneous region of the HCV genome is the hypervariable region-1 (HVR-1), which is typically sequenced and compared between persons in investigations of HCV transmission (25). Sequencing of both HVR-1 and nonstructural protein 5B (NS5B, which encodes an HCV polymerase) can identify genotype and subtype, while sequencing of the 5′ untranslated region (5′-UTR), the most conserved region of the HCV genome, can identify genotype, but is less reliable in subtype classification (24). The HCV HVR-1 was amplified and its intra-host variants (quasispecies) sequenced using 454/Roche GS Junior next-generation sequencing, according to previously described methods (25). When HVR-1 could not be amplified due to low viral titer, recipient NS5B sequences were compared with those identified in the respective organ donor serum, splenocytes or lymphocytes. Phylogenetic analyses (maximum likelihood) were conducted to determine genetic relatedness of HVR-1 quasispecies from recipients. Initial phylogenetic trees were built using the Kimura two-parameter nucleotide substitution model (26). Maximum likelihood phylogenetic trees were constructed using MEGA® (version 5).

Cases were confirmed as donor-derived when, after exclusion of other opportunities for HCV infection, HCV HVR-1 sequences from two or more recipients were genetically related to each other through molecular analyses or when HCV RNA was detected from stored serum obtained from the organ donor in the week preceding death or in the archived splenocyte or lymphocyte specimen obtained from the organ donor at organ recovery.

Results

A timeline of notable findings that informed each transmission investigation is summarized in Figure 1.

Case summaries

Case 1: In 2011, a 25-year-old woman with a 7-year history of active IDU was found unresponsive with hypodermic needles. She developed anoxic encephalopathy and died from heroin overdose. Four days prior to organ recovery, NAT for HCV, HBV and HIV was negative for all three pathogens with the Procleix® Ultrio assay on the Procleix® Tigris system (Novartis, Gen-Probe, San Diego, CA). Heart, liver and both kidneys were recovered and transplanted into four recipients who consented for receipt of organs from an increased risk donor, at two separate transplant centers (27). The liver and right kidney recipients had known HCV infection genotype 3a and 1b, respectively, prior to transplant. Nine days after transplantation, the left kidney recipient was found to have newly detected HCV RNA (viral load: 454 international units [IU] per milliliter [mL]) during routine screening. Thirty-one days after transplantation, the heart recipient at the same institution had detectable HCV RNA (viral load: 38 000 000 IU/mL) during routine screening. Anti-HCV antibody testing was nonreactive for both recipients at the time of the initial PCR result. Neither recipient had other behavioral risk factors for HCV infection nor other healthcare exposures identified as sources of transmission. In a trace back investigation of fresh frozen plasma received by the organ donor, no evidence of HCV infection was identified in one of four plasma donors who were contacted and agreed to re-testing.

HCV RNA was detected in serum obtained after transplantation from all four recipients, but was not detected in the archived donor serum sample or in stored serum from the left kidney and heart recipients obtained 36 and 15 days before transplantation, respectively. HCV RNA was detected in donor splenocytes with a titer of <43 IU/mL. Phylogenetic analysis of the NS5B sequences obtained from the donor, left kidney and heart recipient showed that the donor and both recipients were infected with genetically close HCV genotype 2b strains. HCV HVR-1 quasispecies sequences from the left kidney and heart recipients clustered together in a phylogenetic tree, suggesting a

Figure 1

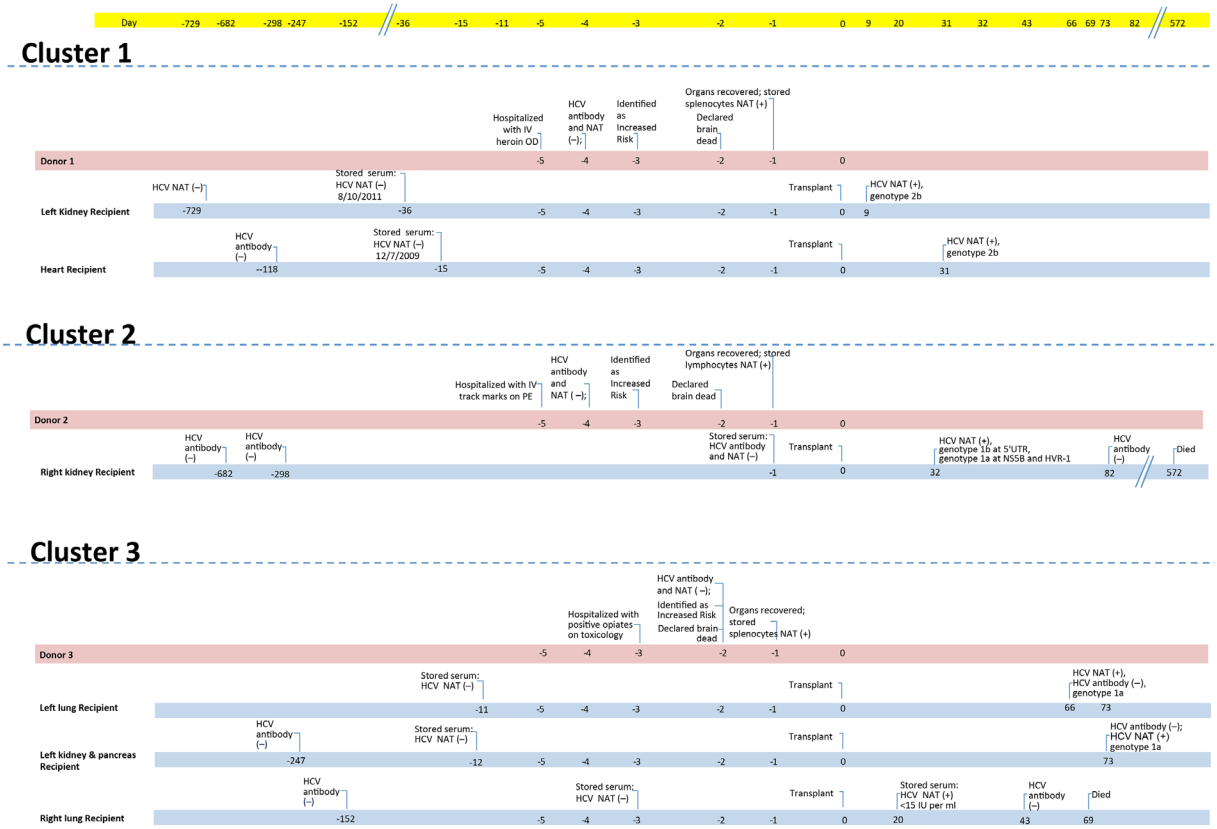

Figure 1: Timeline of notable events informing three investigations of hepatitis C virus (HCV) transmission from increased risk organ donors with negative nucleic acid test (NAT) screening.

common source of infections. The HCV genotype 2b was not detected in the liver or right kidney recipients, who were HCV-infected prior to transplant (Figure 2A).

HCV RNA levels in the heart recipient were nearly 38 million IU/mL 30 days posttransplant, though liver function remained normal. Antiviral treatment was initiated with pegylated interferon (27 weeks postdiagnosis) and ribavirin (16.5 weeks postdiagnosis). After treatment the patient achieved a sustained virologic response and remains free of clinically evident liver disease without graft rejection. The left kidney recipient could not receive interferon therapy due to co-morbidities and had a peak HCV RNA level of >69 million IU/mL approximately 8 months after transplant. After the development of cirrhosis attributed to nonalcoholic steatohepatitis, he began sofosbuvir and ribavirin approximately 2 years after transplantation (Table 1). HCV RNA remains undetectable in the patient.

Case 2: In 2012, a 35-year-old man with history of IDU in the year preceding admission and incarceration in the previous 6 months sustained a motor vehicle accident, resulting in progressive neurologic decline and brain death 4 days after admission. Nonmedical injection sites were noted on his body, which were attributed to active IDU. Predonation infectious disease screening included NAT for HCV, HBV and HIV with the Procleix® Ultrio assay on the Procleix® Tigris system (Novartis, Gen-Probe), which were undetectable on the day before organ recovery. Two organs (left and right kidney) were transplanted into two recipients at the same institution who were not previously HCV-infected (27). Both recipients provided special informed consent given the organ donor's increased risk criteria and were tested for HCV infection following transplantation. Approximately 1 month after transplant, HCV RNA was detected in the right kidney recipient with viral load of 8 000 000 IU/mL with nonreactive anti-HCV antibody. The left kidney recipient had undetectable HCV RNA at 1, 2 and 3 months posttransplant.

No behavioral HCV risk factors were identified in the right kidney recipient. The recipient tested negative by NAT on the day prior to transplant, but received hemodialysis in the week preceding transplant. Infection control breaches were not reported from the hemodialysis center nor were clusters of HCV infection identified among recipients dialyzed on the same day as the right kidney recipient in that center. A trace back of blood products received by the organ donor (2 U of packed red blood cells and fresh frozen plasma) did not identify evidence of transfusion-transmission of HCV.

HCV RNA was not detectable in archived donor serum. However, HCV RNA was detected at low viral load (<43 IU/mL) in an extract of the donor lymphocyte suspension obtained from lymph nodes at organ recovery. HCV from this lymphocyte suspension was classified as belonging to genotype 1b based on HVR-1 sequences. HCV from the right kidney recipient was classified as genotype 1b using the 5′-UTR sequence obtained at the transplant center. At CDC, genotype 1a was identified through sequencing of HVR-1 and NS5B regions. This discrepant genotyping using different genomic regions suggests a potential infection with a recombinant genotype 1a/1b strain, or inaccurate detection of subtypes of genotype 1 using conserved genomic regions such as 5′-UTR. Despite testing an additional recipient serum sample drawn at a later time point after infection was initially identified, HCV HVR-1 sequences from recipient samples did not cluster with HCV HVR-1 sequences from the donor lymphocyte suspension.

Though there was no laboratory evidence of liver dysfunction at the time of HCV detection, the right kidney recipient developed a low level of elevated liver enzymes 4 months following transplant and died 19 months after transplant due to transplant pyelonephritis, sepsis and refusal of dialysis. Autopsy identified chronic cirrhosis presumed due to be steatohepatitis without findings suggestive of hepatitis C–related disease.

Case 3: In 2013, a 38-year-old man with history of IDU sustained severe injuries following an assault and died 1 day after hospital admission. Predonation NAT with the Cobas® TaqScreen MPX v2.0 Test (Roche Molecular Systems) for HCV, HBV and HIV performed on the day of death was negative for all three pathogens. Six organ recipients (left lung, right lung, left kidney/pancreas, right kidney, liver and heart) in three transplant centers received organs after providing informed consent (27). In routine posttransplant screening of the left lung recipient 66 days following transplantation, HCV RNA (>500 000 IU/mL) was detected. The left kidney/pancreas recipient also tested positive for HCV RNA (>14 000 000 IU/mL), 73 days after transplantation. Both patients were infected with HCV genotype 1a strains, with HVR-1 quasispecies clustering in a phylogenetic tree, suggesting a common source of transmission (Figure 2B). HCV RNA was also detected (<15 IU/mL) in stored serum obtained from the donor on the day of death and in stored splenocytes (58 IU/mL) obtained from the donor at organ recovery, 2 days following death. The right lung recipient died shortly after transplantation after developing primary graft dysfunction, progressive interstitial lung disease, pulmonary hemorrhage and cardiac tamponade. However, following identification of infection in the left kidney/pancreas and left lung recipients, stored serum specimens from the right lung recipient obtained 3 days before and 20 days after transplantation were tested. HCV RNA was undetectable in the pretransplant sample but detectable in low levels (<15 IU/mL) in the posttransplant specimen. Genotyping and quasi-species analyses were not performed for these specimens due to insufficient sample quantities. No behavioral risk factors or healthcare exposures were identified as likely sources of transmission. HCV RNA was not detected in the right kidney recipient at 69, 86, 135 and 216 days after

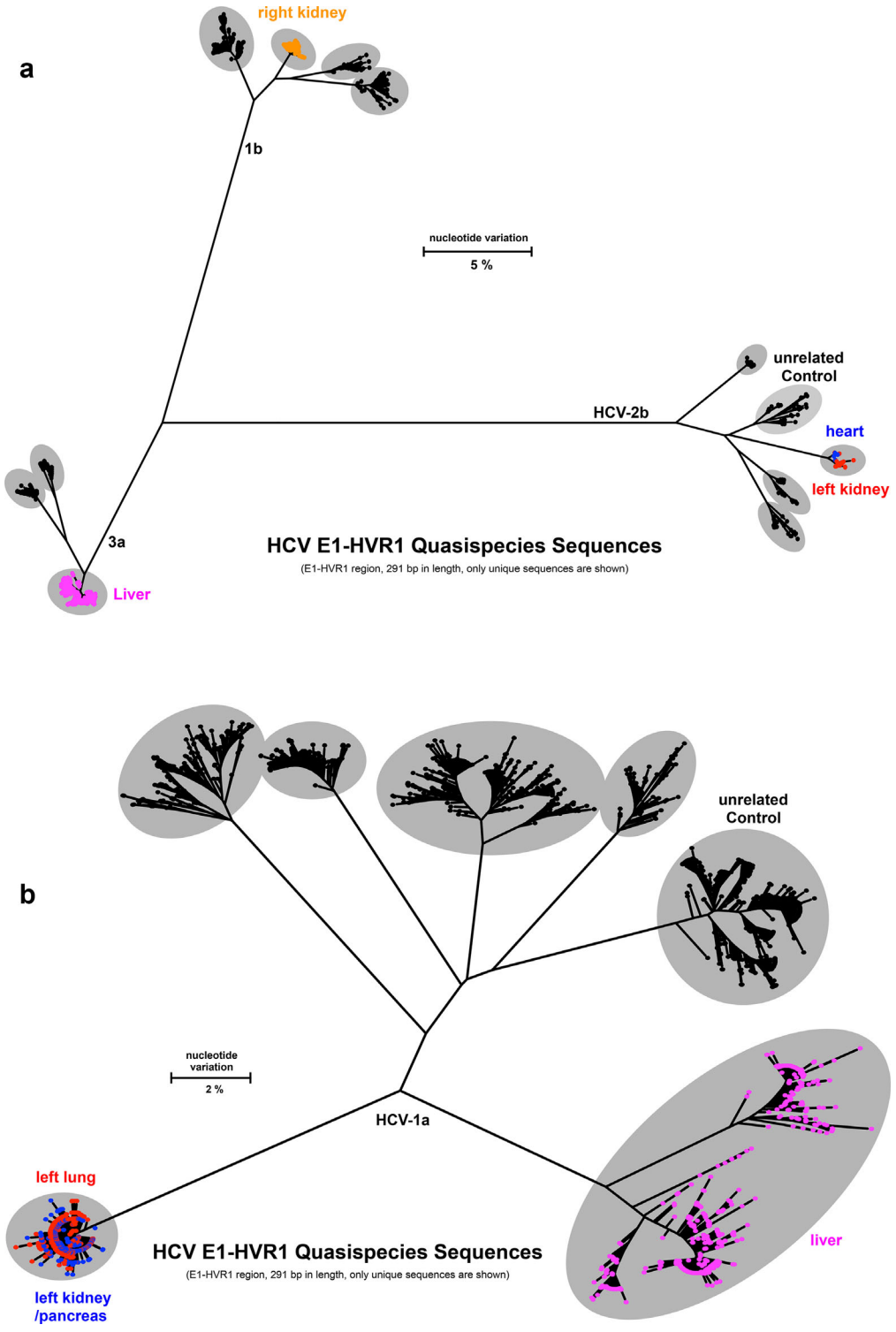

Figure 2: Phylogenetic trees illustrating two clusters of hepatitis C virus (HCV) genetically related at the hypervariable-1 region among organ recipients from common increased risk donors with negative nucleic acid test screening, (A) 2011–2012 and (B) 2013–2014.

Table 1: Clinical characteristics of six cases of donor-derived transmissions of acute hepatitis C virus infections from increased risk donors with negative nucleic acid test screening at four transplant centers in the United States, 2011–2014

	Cluster 1		Cluster 2	Cluster 3		
Characteristics	Left kidney recipient	Heart recipient	Right kidney recipient	Left lung recipient	Left kidney/pancreas recipient	Right lung recipient
Initial presentation	Asymptomatic	Asymptomatic	Asymptomatic	Asymptomatic	Asymptomatic	Asymptomatic
Initial ALT/AST	Normal	Normal	Normal	75/64 IU/mL	Normal	Normal
Significant comorbidities	Severe PVD, depression, hypertension, DM type 2	Congenital heart disease, cardiac arrest with sepsis in 1993	Severe PVD, ESRD, diabetes type II, hypertension, mental retardation	COPD (resolved with transplant)	Diabetes type 1 (resolved with transplant), ESRD (resolved with transplant), hypertension	CAD, hypertension, diabetes type II, interstitial lung disease
Postoperative immunosuppressive medication regimen	Thymoglobulin, tacrolimus, prednisone, mycophenolate mofetil	Mycophenolate mofetil, prednisone, tacrolimus	Alemtuzumab, mycophenolate mofetil, tacrolimus, methylprednisolone, prednisone	Basiliximab, mycophenolate mofetil, tacrolimus, prednisone	Thymoglobulin, sirolimus, cyclosporine	Tacrolimus, mycophenolate mofetil, prednisone
Viral load at diagnosis (IU/mL)	454	37 748 148	8 913 858	534 164	14 385 443	<15
Peak viral load (IU/mL)	37 748 148	>69 million	31 641 506	629 778	14 385 443	<15
Antiviral treatment (week initiated)	Sofosbuvir, ribavirin (115 weeks)	Pegylated interferon (27 weeks), ribavirin (16.5 weeks)	Not given	Simeprivir sofosbuvir (16 weeks)	Not given	Not given
SVR	TBD	Yes	No*	TBD	No*	No*
Posttransplant clinical course	Compensated cirrhosis (biopsy: steatohepatitis)	No liver disease	Died from sepsis, ESRD, compensated cirrhosis (biopsy: steatohepatitis)	No liver disease	No liver disease	Died from transplant complications

*These recipients were not treated. Thus SVR could not be measured or achieved.

ALT/AST, alanine aminotransferase/aspartate aminotransferase; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; ESRD, end-stage renal disease; PVD, peripheral vascular disease; SVR, systemic vascular resistance.

transplantation. HCV RNA was not detected in the heart recipient at 6-month follow-up. The liver recipient had known genotype 1a HCV infection prior to transplantation.

The left lung recipient had mild elevation of liver transaminases (alanine aminotransferase [ALT] of 75 IU/mL and aspartate aminotransferase [AST] of 64 IU/mL) at the time of infection detection, without further deterioration and with resolution on repeat testing. The left kidney/pancreas recipient was asymptomatic at diagnosis and had no evidence of liver injury at 7-month follow-up.

Discussion

Based on OPTN data as of December 5, 2014, 4.4% of deceased donors with organs recovered for transplantation in 2013 had a positive HCV antibody test. The residual risk of HCV infection from increased risk donors with negative serologic screening has been estimated from 0.26 (hemophiliacs) to 300.6 (IDU) per 10 000 donors (28). PHS guidelines, therefore, have recommended NAT screening of donors for HCV in addition to mandated serology, in recognition of the prolonged time (up to 10 weeks) for seroconversion and the increased yield from NAT, which shortens the window period to 7–10 days (15–18).

US and Canadian subject matter experts recommend that recipients of organs from increased risk donors should be screened for HCV, HIV, (both with NAT) and HBV (with surface antigen and core antibody testing) early after transplant, at periodic intervals such as 1 month, 3 months, 6 months and 1 year after transplant (18). All recipients in these investigations were consented for receipt of organs from increased risk donors and all were screened for HCV with NAT, from 9 to 73 days posttransplant. Surveys suggest that adherence to these guidelines is incomplete. Among surveyed US transplant infectious disease physicians, 8% of respondents reported not performing any informed consent prior to transplant, 25% reported not obtaining serology and 35–43% reported not obtaining NAT for HIV, HBV and HCV following transplant of organs from increased risk donors (29).

We describe the first published report of transmissions of HCV from increased risk donors with negative NAT screening to recipients of solid organs through transplantation. Evidence for donor-derived transmission is especially compelling when taken together with other lines of evidence suggesting transmission, including the history of donor IDU in the 12 months preceding hospitalization; evidence suggesting active donor IDU just preceding hospitalization in each donor; genetically related HCV HVR-1 sequences among at least two recipients in two of three clusters; undetectable HCV RNA in recipient serum collected just before death in two of three clusters and detection of HCV RNA in splenocytes or lymphocytes recovered from all three donors at organ recovery. Posttransplant follow-up with NAT screening was critical to identifying donor-derived HCV as evidenced by all three investigations where routine NAT confirmed transmission in recipients with minimal to no clinically evident liver disease and negative serologic testing when performed.

In each cluster, the identification of HCV in stored samples derived from donor immunologic tissue obtained at organ procurement was an important piece of evidence used to establish donor-derived transmission. Such cells (derived from either lymph nodes or spleen) are available routinely after transplantation because they are typically recovered and stored for potential histocompatibility testing by transplant centers. Tissue typing laboratories follow strict precautions to avoid HCV cross contamination and, thereby, false positive NAT results of stored splenocytes and lymphocytes. Although low levels of detectable HCV could theoretically result from nonviable genetic elements related to prior exposure and unsuccessful infection, this seems unlikely given the compelling epidemiologic evidence suggesting donor-derived transmission and the negative donor serology for HCV. To our knowledge, this is the first use of stored samples derived from immunologic tissue containing peripheral blood mononuclear cells for purposes of establishing HCV transmission, and offers promise for similar testing in future donor-derived infection investigations.

Notably, transmission of HCV was not universal among organ recipients from HCV-infected donors in these three clusters, in contrast to findings from other investigations (7–9). In one study, of 29 organ recipients from anti-HCV positive donors, 96% had detectable HCV RNA in recipient sera (30). Likewise, in three previously published investigations describing unexpected HCV transmission from seronegative donors, HCV transmission was noted in all nine organ recipients who did not have known HCV infection before transplantation (7–9). In a 2011 investigation of donor-derived HBV infection, only three of five recipients acquired HBV from a donor with low level HBV by NAT, despite negative donor serologic and risk factor screening (31). In the three clusters that we summarize, at least 3 of 12 recipients did not develop HCV infection when tested up to 7 months after transplantation and in two of the clusters there was discordant transmission among kidney recipients from the same donor. These findings suggest that recipient host factors other than the organ itself, such as the choice of immunosuppressive regimens and other host and organ-specific factors, might impact the likelihood of transmission. It is currently unknown whether immunosuppressive regimens should be altered when transplanting organs from increased risk donors. Also, the comparatively lower transmissibility of HCV from these donors might be related to the low viral load observed in donor samples presumably due to very recent infection prior to death.

Even with very sensitive testing assays and donor risk assessments for HCV, HBV and HIV, it is impossible to fully

prevent transmission. In some cases, HCV risk factors might be undetected due to the inherent limitations of next-of-kin donor history. In all three investigations, donor IDU in the previous 12 months was identified by next-of-kin history, but history suggesting active IDU was less direct. One donor was identified with active IDU (heroin overdose at death), while the other two had more subtle evidence suggesting very recent IDU—one from physical examination suggesting active IDU and the other with positive toxicology for opiates. Moreover, other less common risk factors for very recent HCV transmission are likely missed in next-of-kin history, such as history of men having sex with men. The inherent testing limitations need to be clearly explained to recipients of these increased risk donors, underscoring the importance of routine informed consent and early posttransplant testing with NAT in all recipients of solid organs from increased risk donors. These steps were taken in each investigation, even though two of three predated release of revised PHS guidelines.

The transmission of HCV through solid organ transplantation has significant implications for posttransplant morbidity and mortality. A multicenter cohort study examining registered cardiac transplantations over a 10-year period found that receipt of HCV-positive donor hearts was associated with increased mortality, independent of recipient pretransplant HCV status or recipient age (10). Likewise, HCV infection in recipients of HCV-positive kidneys is associated with worse patient and graft survival (11–14). Risk–benefit analysis for kidney transplant candidates is especially important given that continued dialysis for end organ failure is an alternative option. Moreover, when transmission occurs, recognition is critical. Early treatment after transplant may effectively eradicate HCV infection before the development of chronic liver disease. The heart recipient in cluster 1 was successfully treated and achieved sustained virologic response without allograft rejection, despite the use of an interferon-based regimen. Given the adverse impact of HCV infection on posttransplant outcomes, particularly in the context of sustained immunosuppression, further study is warranted to determine whether newly infected recipients should be prioritized for antiviral therapy, especially given the increased availability of interferon-sparing direct-acting antiviral regimens (32).

There were several limitations to our report. First, donor HCV HVR-1 sequences could not be definitely linked to recipients' HVR-1 sequences, which are typically compared in transmission investigations (25). The low titers of HCV RNA detected in donor samples, while precluding HVR-1 analysis, serve as compelling evidence of very recent donor infection. Second, molecular evidence of transmission could not be established in one investigation. However, other lines of evidence supporting donor-derived transmission were strong—HCV RNA was detected from donor lymphocytes obtained at organ recovery but was not detected in serum collected on the day preceding death, and epidemiologic investigation did not identify any other compelling cause. While the right kidney recipient's infection theoretically could have been acquired from hemodialysis immediately preceding transplant, the case for donor-derived transmission was far stronger given the detection of HCV in donor lymphocytes, the donor's high-risk status, and the absence of infection control breaches or HCV clusters at that hemodialysis center. Moreover, the discordant genotype findings in testing of donor lymphocytes and recipient serum in the same cluster might be explained by a recombinant HCV genome in the donor or recipient (33), or might be a result of different HCV genotype detected in peripheral blood mononuclear cells versus that detected intra-host in serum, as previously described (34). Third, blood product trace back was incomplete due to incomplete voluntary follow-up from blood product donors. However, the residual risk of HCV transmission from blood donors is estimated at less frequent than 1:1 000 000 (35–36) and given the screening of blood donors with risk factor assessment, HCV serology and HCV NAT, the risk of HCV associated with active donor IDU preceding death is much greater than the risk of acquisition from blood products. Finally, active IDU was only definitively identified in the first cluster. Nonetheless, other findings from clinical care are compelling for active IDU by the donors in the other two clusters.

In summary, HCV may be transmitted from organ donors to recipients even when the donors test negative by NAT. In all likelihood, this reflects the eclipse phase infection in donors with increased risk behaviors for the acquisition of HCV infection, especially injection drug users (17). Overall this risk is low and the use of the increased risk donors may be warranted given the younger age and better organ quality of some of these donors. Nevertheless, it is important to ensure that recipients understand the limitations of current testing strategies and the potential for transmission despite negative NAT. Early posttransplant screening of recipients with NAT for HCV RNA, along with protocols for monitoring and reporting of newly detected infections, are critical to identifying patients with new infections who may benefit from antiviral therapies.

Acknowledgments

We thank the numerous transplant coordinators, clinical providers and support staff from institutions involved in these public health investigations for their assistance in clinical management, laboratory testing and data aggregation. Financial support: The Centers for Disease Control and Prevention.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*. The findings and conclusions in this report are those of the author(s) and do not necessarily represent

the official position of the Centers for Disease Control and Prevention.

References

1. Ison MG, Grossi P. The AST Infectious Diseases Community of Practice. Donor-derived infections in solid organ transplantation. Am J Transplant 2013; 13: 22–30.
2. Health Resources Services Administration, US Department of Health & Human Services. Uniting people and information to help save lives [cited 30 Jul 2014]. Available from: http://optn.transplant.hrsa.gov/.
3. Shafer TJ, Wagner D, Chessare J, et al. US Organ Donation breakthrough collaborative increases organ donation. Crit Care Nurs Q 2008; 31: 190–210.
4. Harring TR, O'Mahony CA, Goss JA. Extended donors in liver transplantation. Clin Liver Dis 2011; 15: 879–900.
5. Lonze BE, Dagher NN, Liu M, et al. Outcomes of renal transplants from Centers for Disease Control and Prevention high-risk donors with prospective recipient viral testing: A single-center experience. Arch Surg 2011; 146: 1261–1266.
6. Kucirka LM, Sarathy H, Govindan P, et al. Risk of window period hepatitis-C infection in high infectious risk donors: Systematic review and meta-analysis. Am J Transplant 2011; 11: 1188–2000.
7. Centers for Disease Control and Prevention. Transmission of hepatitis C virus through transplanted organs and tissue—Kentucky and Massachusetts, 2011. MMWR Morb Mortal Wkly Rep 2011; 60: 1697–1700.
8. Ison MG, Llata E, Conover CS, et al. Transmission of human immunodeficiency virus and hepatitis C virus from an organ donor to four transplant recipients. Am J Transpl 2011; 11: 1218–1225.
9. Tugwell BD, Patel PR, Williams IT, et al. Transmission of hepatitis C virus to several organ and tissue recipients from an antibody-negative donor. Ann Intern Med 2005; 143: 648–654.
10. Gasink LB, Blumberg EA, Localio AR, Desai SS, Israni AK, Lautenbach E. Hepatitis C virus seropositivity in organ donors and survival in heart transplant recipients. JAMA 2006; 296: 1843–1850.
11. Fabrizi F, Martin P, Dixit V, Messa P. Meta-analysis of observation studies: Hepatitis C and survival after renal transplant. J Viral Hepat 2014; 21: 314–324.
12. Carbone M, Mutimer D, Neuberger J. Hepatitis C virus and nonliver solid organ transplantation. Transplantation 2013; 95: 779–786.
13. Mendez R, El-Shahawy M, Obispo E, et al. Four years follow-up of hepatitis C positive kidneys into hepatitis C negative recipients—Prospective study. J Am Soc Nephrol 1995; 6: 1105.
14. Fabrizi F, Martin P, Ponticelli C. Hepatitis C virus infection and renal transplantation. Am J Kidney Dis 2001; 38: 919–934.
15. Seem DL, Lee I, Umscheid CA, Kuehnert M. PHS guideline for reducing human immunodeficiency virus, hepatitis B virus, and hepatitis C virus transmission through organ transplantation. Public Health Rep 2013; 128: 247–343.
16. Kleinman SH, Lelie N, Busch MP. Infectivity of human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus and risk of transmission by transfusion. Transfusion 2009; 49: 2454–2489.
17. Busch MP, Glynn SA, Wright DJ, et al. Relative sensitivities of licensed nucleic acid amplification tests for detection of viremia in early human immunodeficiency virus and hepatitis C virus infection. Transfusion 2005; 45: 1853–1863.
18. Humar A, Morris M, Blumberg E, et al. Nucleic acid testing (NAT) of organ donors: Is the ''best'' test the right test? A consensus conference report. Am J Transplant 2010; 10: 889–899.
19. Organ Procurement and Transplantation Network. Organ Procurement and Transplantation Network Policies [cited 5 June 2014]. Available from: http://optn.transplant.hrsa.gov/ContentDocuments/OPTN_Policies.pdf#nameddest=Policy15.
20. Ison MG, Hager J, Blumberg E, et al. Donor-derived disease transmission events in the United States: Data reviewed by the OPTN/UNOS Disease Transmission Advisory Committee. Am J Transplant 2009; 9: 1929–1935.
21. Centers for Disease Control and Prevention. Viral hepatitis case report [cited 5 Dec 2014]. Available from: http://www.cdc.gov/hepatitis/PDFs/vhsp02.pdf.
22. Centers for Disease Control and Prevention. Viral hepatitis outbreaks: Healthcare investigation guide [cited 3 June 2014]. Available from: http://www.cdc.gov/hepatitis/Outbreaks/HealthcareInvestigationGuide.htm.
23. Mixson-Hayden T, Lee D, Ganova-Raeva L, et al. Hepatitis B virus and hepatitis C virus infections in United States-bound refugees from Asia and Africa. Am J Trop Med Hyg 2014; 90: 1014–1020.
24. Smith DB, Bukh J, Kuiken C, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: Updated criteria and genotype assignment web resource. Hepatology 2014; 59: 318–327.
25. Dimitrova Z, Campo DS, Ramachandran S, et al. Evaluation of viral heterogeneity using next-generation sequencing, end-point limiting-dilution and mass spectrometry. In Silico Biol 2011–2012; 11: 183–192.
26. Kumar S, Tamura K, Nei M. MEG A3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 2004; 5: 150–163.
27. Centers for Disease Control and Prevention. Guidelines for preventing transmission of human immunodeficiency virus through transplantation of human tissue and organs. MMWR Recomm Rep 1994; 43: 1–17.
28. Kucirka LM, Sarathy H, Govindan P, et al. Risk of window period hepatitis-C infection in high infectious risk donors: Systematic review and meta-analysis. Am J Transplant 2011; 11: 1188–1200.
29. Theodoropoulos N, Ladner DP, Ison MG. Screening recipients of increased-risk donor organs: A survey of transplant infectious diseases physician practices. Transpl Infect Dis 2013; 15: 545–549.
30. Pereira BJ, Wright TL, Schmid CH, Levey AS. A controlled study of hepatitis C transmission by organ transplantation. The New England Organ Bank Hepatitis C Study Group. Lancet 1995; 345: 484–487.
31. Centers for Disease Control and Prevention. Notes from the field: Transplant-transmitted hepatitis B virus—United States, 2010. MMWR Morb Mortal Wkly Rep 2011; 60: 1087.
32. Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. N Engl J Med 2013; 368: 1907–1917.
33. Shi W, Freitas IT, Zhu C, et al. Recombination in hepatitis C virus: Identification of four novel naturally occurring inter-subtype recombinants. PLoS ONE 2012; 7: e41997. doi: 10.1371/journal.pone.0041997.
34. Roque-Afonso AM, Ducoulombier D, Di Liberto G, et al. Compartmentalization of hepatitis C virus genotypes between plasma and peripheral blood mononuclear cells. J Virol 2005; 79: 6349–6357.
35. Zou S, Stramer SL, Dodd RY. Donor testing and risk: Current prevalence, incidence, and residual risk of transfusion-transmissible agents in US allogeneic donations. Transfus Med Rev 2012; 26: 119–128.
36. Zou S, Dorsey KA, Notari EP, et al. Prevalence, incidence, and residual risk of human immunodeficiency virus and hepatitis C virus infections among United States blood donors since the introduction of nucleic acid testing. Transfusion 2010; 50: 1495–1504.