American Journal of Transplantation 2015; 15: 555–559
Wiley Periodicals Inc.

doi: 10.1111/ajt.12986

Case Report

Laboratory Identification of Donor-Derived Coxsackievirus B3 Transmission

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Unexpected donor-to-recipient infectious disease transmission is an important, albeit rare, complication of solid organ transplantation. Greater work and understanding about the epidemiology of these donor-derived transmissions is continually required to further mitigate this risk. Herein we present the first reported case of proven donor-derived transmission of coxsackievirus serogroup-3, an enterovirus, following solid organ transplant. Swift and effective communication between the organ donation agency, treating physicians, laboratory testing and notification ensured a coordinated approach. The resulting clinical syndromes in the organ recipients were mild. This case highlights the requirement for ongoing surveillance over a broad range of infecting pathogens that may present as a donor-derived infection.

Abbreviations: ALT, alanine aminotransferase; BAL, bronchoalveolar lavage; CMV, cytomegalovirus; ct, cycle threshold; CVA, coxsackievirus group A; CVB, coxsackievirus group B; CVB3, coxsackievirus group B, serotype 3; DTAC, Disease Transmission Advisory Committee; EBV, Epstein-Barr virus; EV, enterovirus; HHV-8, human herpesvirus 8; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; ICU, intensive care unit; ND, not detected; PCR, polymerase chain reaction; rt-PCR, real-time PCR; UNOS/OPTN, United Network for Organ Sharing/Organ Procurement and Transplantation Network; VF, ventricular fibrillation; VZV, varicella zoster virus

Received 23 June 2014, revised 04 August 2014 and accepted for publication 19 August 2014

Introduction

Enteroviruses (EVs) are single stranded, positive sense RNA viruses in the family *Picornaviridae*. The EV genus is split into four distinct human species (human EV A, B, C and D). Within each species, based on molecular and biologic characteristics, individual serotypes maintain their traditional names such as coxsackievirus, divided into groups A and B, poliovirus and echovirus, as well as numbered strains, such as EV-A71 (1). These small RNA viruses are quite stable in liquid environments and can survive for many weeks in water, body fluids and sewage. They are ubiquitous agents found worldwide. Group A coxsackieviruses (CVA), which are split between the species EV-A, -B and -C, are the predominant EV subgroup associated with viral encephalitis, while group B coxsackieviruses (CVB), contained within the species EV-B, are an important cause of viral myocarditis, dilated cardiomyopathy and aseptic meningitis (2,3). There are six serotypes of CVB, and serotype 3 appears to have an additional tissue tropism for the liver and pancreas (4).

We describe the first report of donor-derived transmission of an EV by solid organ transplant, where the donor and two of the three evaluable organ recipients were all shown to be viremic with the same coxsackievirus serotype 3 (CVB3). Both recipients had detectable virus in other specimens, including feces, rectal and throat swabs. Clinical consequences for the infected organ recipients appear to have been relatively mild.

Case Report

The deceased donor, a young adult resident in Australia, without any significant past medical history, died in the springtime of 2011 following a subarachnoid hemorrhage associated with a ventricular fibrillation cardiac arrest after 10 days of headache culminating in collapse, but no fever. Other symptoms in the preceding weeks included nonspecific malaise and altered mood. The liver, kidneys, pancreas and lungs were procured for transplantation after development of brain death and with family consent. The heart was

not accepted for whole organ transplantation due to poor cardiac function. Transthoracic echocardiogram had revealed mild to moderate impairment of the left ventricle systolic function, with severe hypokinesis of the basal half, to two-thirds of the left ventricle, and akinetic septal, antero-septal and inferior segments. The procured heart valves were stored by the tissue bank and underwent routine histopathology and microbiological testing. Donation resulted in transplantation of the liver (recipient 1), left kidney/pancreas (recipient 2), lungs (recipient 3) and right kidney (recipient 4), which occurred in different Australian states from the donor.

Investigations for a possible donor-derived infection were prompted clinically, by the development of elevated transaminases in the liver recipient, and diagnostically, by the findings of a significant neutrophilic pericarditis, myocarditis and perivascular infiltration on the histopathological sections from the explanted heart valves. Stored plasma, serum, heart valve tissue and processing solutions from the donor were retrospectively recovered and tested. EV was detected by polymerase chain reaction (PCR) in two sera, one of the two plasma samples and in the initial heart collection solution (see Table 1). Herpes viruses (herpes simplex virus types 1 and 2 (HSV-1, HSV-2), cytomegalovirus (CMV), and varicella zoster virus and adenovirus were negative by PCR.

The liver recipient, transplanted for acute fulminant hepatic failure secondary to a presumed adverse drug reaction, developed a desquamating rash, markedly elevated alanine aminotransferase (ALT) greater than 2000 IU/L (normal range <45 IU/L), an elevated troponin I to 0.31 μg/L (normal range <0.04 μg/L) and a small pericardial effusion, in the first few days following transplant. Immunosuppressive medications included basiliximab induction, tacrolimus, azathioprine and steroids. A liver biopsy performed at day 5 posttransplant showed predominantly lobular changes with pan-acinar disarray, numerous acidophic hepatocytes and acidophilic bodies and small areas of confluent necrosis with a moderate lobular mononuclear inflammatory infiltrate. Eosinophils were not prominent. Immunohistochemical stains for HSV-1, HSV-2, CMV, adenovirus, Epstein–Barr virus and human herpesvirus 8 were negative. EV-specific stains were not available. The differential diagnosis included viral hepatitis, ischemia, drug reaction and antibody mediated rejection. Plasma exchange and intravenous gamma globulin were initially commenced as treatment for possible antibody mediated rejection. Antibiotics (doxycycline, vancomycin and ciprofloxacin) were also included to empirically cover bacterial pericarditis. Subsequent PCR testing on serum, throat swab and feces were positive for both EV and HSV-1. Intravenous acyclovir, 10 mg/kg 8 hourly, was commenced. The patient improved clinically and was discharged from the intensive care unit, 10 days after transplantation, with an ALT in the normal range.

Following the results generated from the first recipient and the donor, recipient 2, who received a combined kidney/pancreas transplant with immunosuppressant medications including basiliximab induction, tacrolimus, mycophenolate mofetil and steroids, underwent investigations where EV was detected by PCR in plasma, feces, rectal swab and throat swab. HSV-2 was also detected from the rectal swab. HSV-1 was not detected in any of the samples. Despite a subsequent brief episode of fever, vomiting and diarrhea 2 weeks posttransplant, for which the patient received intravenous immunoglobulin for its potential antiviral effects, repeat plasma, throat and rectal swab investigations at that time were negative for EV and the patient recovered quickly with excellent function of both organs. Recipient 3, who received a bilateral sequential lung transplant for end-stage lung disease secondary to cystic fibrosis, received basiliximab induction, then tacrolimus, azathioprine and steroids for immunosuppression, had an uneventful clinical course posttransplant, and throat swab, rectal swab, plasma and bronchoalveolar lavage (BAL) specimens were negative for both EV and HSV. She was discharged from hospital 2 weeks after transplantation. The treating team caring for recipient 4, who received the right kidney, was notified of the EV infection of the donor and recipient 1, but elected to not undertake further testing as the patient was asymptomatic, and no follow-up information was available. The stored heart valve tissue from the donor was not used. Pretransplant sera from the three evaluable recipients were negative for EV when retrospectively tested by PCR.

The laboratory used real-time (rt) TaqMan PCR for EV detection (primers and probe targeting 5′ UTR) (5) and HSV-1 and -2 detection (targeting glycoprotein-B, modified from Druce et al ([6])). PCR was run for 45 cycles. The cycle threshold (ct) values at which EV and HSV were detected in the individual samples are presented in Table 1. Where there was sufficient amplified product, positive EV detections were genotyped using conventional PCR and sequenced targeting VP1 (5). All sequenced EV PCR products from the donor and two recipients were identified as CVB3. Of the circulating EV strains that were sequenced in the same year, CVB3 accounted only for 8.7% of strains (10 out of 115 EV strains). In order to demonstrate the molecular relatedness of the CVB3 strains from the donor and the two recipients, a phylogenetic analysis compared these strains with all sequenced CVB3 strains from 2008–2013 (Figure 1). The CVB3 strains isolated from the donor and the two recipients were genetically identical and distinct from other circulating CVB3 strains. This analysis and the fact that the recipients were geographically separate from the donor, provides strong evidence for donor-derived infection rather than community-acquired infection.

Discussion

A wide range of infections have been recognized to be transmitted from donor to recipient through organ

Table 1: Investigation results of enterovirus and herpes simplex virus laboratory testing from the donor and recipients

	Timing of sample	Sample type	EV rt-PCR (ct)	HSV rt-PCR (ct)
Donor	Predonation	Serum	Detected (35)	Not detected
			Detected (40)	Not detected
		Plasma	Detected (40)	Not detected
			Not detected	Not detected
	Postmortem	Heart[1]	Detected (38)	Not detected
Recipient 1 (liver)	Pretransplant	Serum	Not detected	HSV-1 detected (41)
	5 days posttransplant	Serum	Detected (27)	HSV-1 detected (30)
		Liver biopsy[2]	Not detected	Not detected
	7 days posttransplant	Throat swab	Detected (43)	HSV-1 detected (20)
	8 days posttransplant	Feces	Detected (39)	HSV-1 detected (31)
	13 days posttransplant	Liver biopsy[3]	Not detected	Not detected
	17 days posttransplant	Serum	Inhibited	–
		Feces	Not detected	–
	18 days posttransplant	Throat swab	Not detected	–
	20 days posttransplant	Plasma	Not detected	–
Recipient 2 (kidney/pancreas)	Pretransplant	Serum	Not detected	Not detected
	7 days posttransplant	Plasma	Detected (36)	Not detected
		Throat swab	Not detected	Inhibited
		Rectal swab	Detected (36)	HSV-2 detected (24)
	8 days posttransplant	Plasma	Detected (37)	Not detected
		Feces	Detected (31)	Not detected
	17 days posttransplant	Plasma	Not detected	–
		Throat swab	Not detected	–
		Rectal swab	Not detected	–
	18 days posttransplant	Feces	Inhibited	–
Recipient 3 (lungs)	Pretransplant	Serum	Not detected	Not detected
	7 days posttransplant	Throat swab	Not detected	Not detected
		Rectal swab	Not detected	Not detected
	8 days posttransplant	Plasma	Not detected	Not detected
	10 days posttransplant	BAL	Not detected	Not detected

EV, enterovirus; HSV, herpes simplex virus; rt-PCR, real-time polymerase chain reaction; ct, cycle threshold; BAL, bronchoalveolar lavage; —, not tested.
[1]PCR positive from the collection solution; negative from aortic and pulmonary tissues and solutions, rinse solution, freeze solution and tissue trimmings (i.e. one positive out of nine samples tested).
[2]Paraffin-embedded tissue sample.
[3]Fresh tissue sample.

transplantation (7), although EV transmission has not been reported. Transplantation of organs from deceased donors who had fever or viral syndromes is controversial, prompting the need for improved microbiologic screening tools (8). Despite logistical constraints, the follow up and microbiological testing of the multiple organ recipients from the common donor was relatively swift and effective in this case.

Cases of donor-derived disease transmission are classified as either proven, probable or possible, based on the *ad hoc* United Network for Organ Sharing/Organ Procurement and Transplantation Network Disease Transmission Advisory Committee (7,9). Our case meets the classification as a proven case of donor-derived CVB3 transmission, namely meeting the following conditions:

- There was a suspected transmission event.
- Laboratory evidence of CVB3 in the organ recipient.
- Laboratory evidence that the same, genetically identical virus identified in another organ recipient and from the original common donor.
- Pretransplant laboratory evidence that the recipients did not have CVB3.

Cardiotropic viruses, especially CVB and adenoviruses, and to a lesser extent, human CMV and parvovirus B19, have been detected from a high proportion of myocardial tissue samples from heart (10) and heart valve donors (11), suggesting a significant risk for viral transmission by cardiovascular allografts. The clinical significance of such a transmission is suggested by the association of pediatric cardiac graft loss with the presence of viral genomes in the heart tissue (12) and reports of EV infection as an etiological cause for unexplained late severe adverse cardiac events in the adult heart-transplant population (13). EV viremia has also been reported in Scottish blood donors, at a frequency

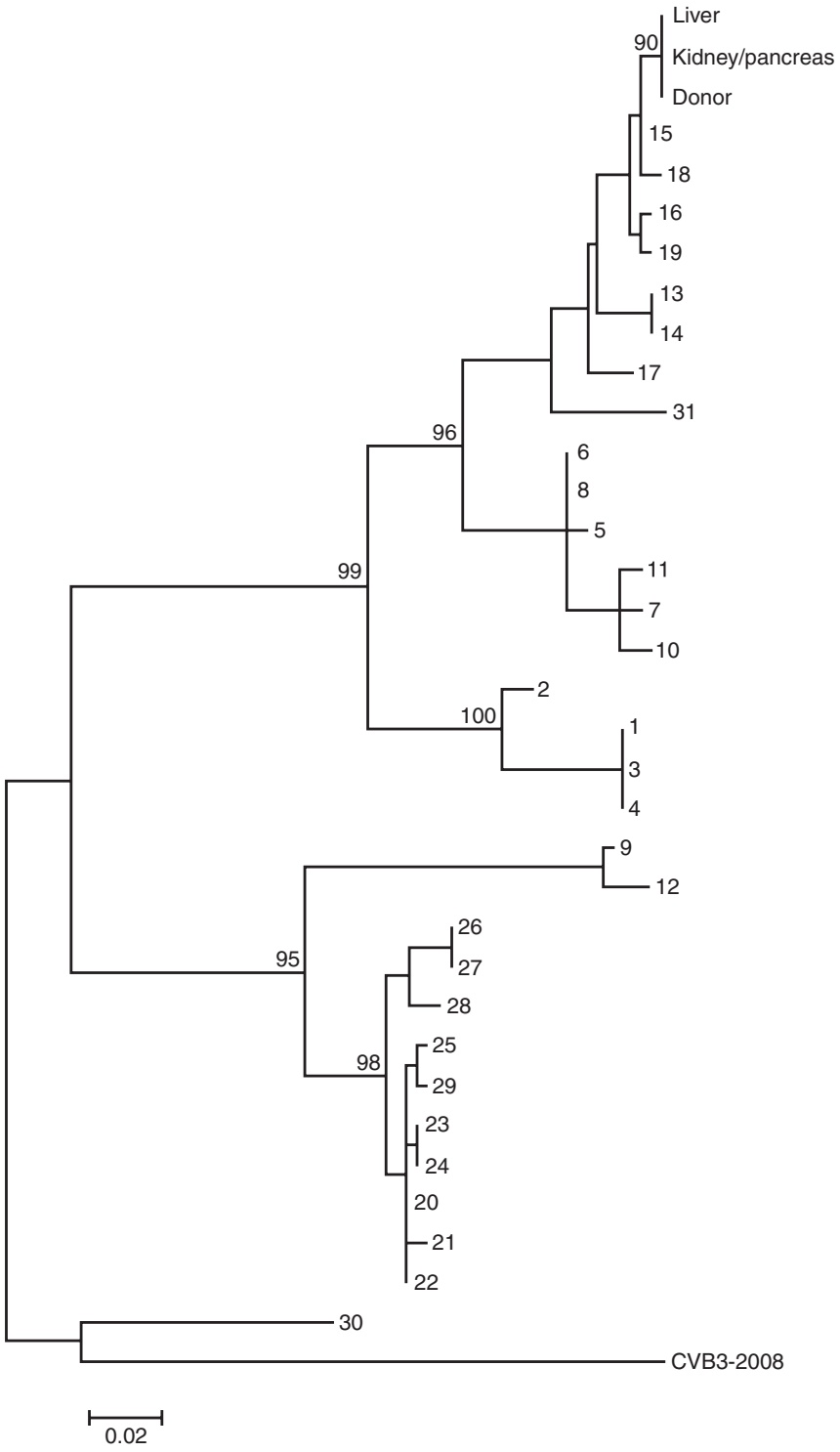

Figure 1: Phylogentic clustering of donor and recipient coxsackievirus B3. Maximum-likelihood tree depicting the relationships between CVB3 VP1 sequences from the donor, kidney/pancreas recipient and liver recipient, and 31 unrelated clinical strains collected over the period 2008–2013 (1–31). The tree was constructed by maximum-likelihood methods using the Tamura-3-parameter model of evolution based on analysis of 344 nucleotide VP1 sequences. The coxsackievirus-B3 prototype strain CVB3-2008 was included in the analysis and used as an out-group to root the tree. Bootstrap values greater than 90% are indicated. The clustering of the CVB3 strains from the donor and the two organ recipients indicates molecular evidence of genetic relatedness, distinct from other sequenced circulating CVB3 strains.

of approximately 1 in 4400 donors, which increases to 1 in 950 in the early winter months (October) (14).

Given that CVB3 is an important cause of viral myocarditis, it is plausible that the donor in this case had an unrecognized CVB3-related myocarditis, suggested by the reduced cardiac function premortem and subsequent demonstration of inflammation on the postmortem histopathology of the heart. This virus was then transmitted to the organ recipients. The liver recipient developed a severe hepatitis with a histological appearance in keeping with acute viral infection and most likely due to EV rather than HSV-1 reactivation given the negative HSV-1 immunohistochemistry. In contrast, there was a relatively mild clinical syndrome in the kidney/pancreas recipients, and incomplete transmission to the lung recipient. This variation in clinical severity of disease, is likely to be related to the inoculum and tissue tropism of CVB3 (4,15), the organ transplanted and the type of immune suppression used.

A coordinated and systematic approach between the laboratory and the different transplant units is required when investigating possible low frequency, ''near miss'' transmission events like this, especially given that the clinical consequences of a CVB3 donor-derived infection appears to be mild. A different clinical outcome, however, may occur in the setting of alternative organ procurement, especially heart transplantation. Furthermore, donor-derived transmission of another species of EV, with different tissue tropism, may have distinct and more severe clinical consequences. Given the impracticality of screening donors for all possible infections, transplant clinicians should consider and test for EVs when investigating cases of possible donor-derived infections.

Acknowledgments

The authors acknowledge the work performed by the Australian DonateLife Network.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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