American Journal of Transplantation 2011; 11: 2715–2723
Wiley Periodicals Inc.

doi: 10.1111/j.1600-6143.2011.03769.x

Primary and Reactivated HHV8 Infection and Disease After Liver Transplantation: A Prospective Study

G. Pietrosi[a,*], G. Vizzini [a], L. Pipitone[b], G. Di Martino[c], M. I. Minervini[b], G. Lo Iacono[c], P. G. Conaldi[c], P. Grossi[d], V. Lamonaca[e], L. Galatioto[a], S. Gruttadauria[f] and B. Gridelli[f]

Departments of [a]Gastroenterology and Hepatology, [b]Pathology, [c]Microbiology and Virology, [e]Infectious Diseases and [f]Abdominal Surgery, Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione (ISMETT), University of Pittsburgh Medical Center, Palermo, Italy
[d]Department of Infectious and Tropical Diseases, University of Insubria, Varese, Italy
**Corresponding author: Giada Pietrosi, gpietrosi@ismett.edu*

Human herpesvirus 8 (HHV8) is pathogenic in humans, especially in cases of immunosuppression. We evaluated the risk of HHV8 transmission from liver donors, and its clinical impact in southern Italy, where its seroprevalence in the general population is reported to be as high as 18.3%. We tested 179 liver transplant recipients and their donors for HHV8 antibodies at the time of transplantation, and implemented in all recipients a 12-month posttransplant surveillance program for HHV8 infection. Of the 179 liver transplant recipients enrolled, 10.6% were HHV8 seropositive before transplantation, whereas the organ donor's seroprevalence was 4.4%. Eight seronegative patients received a liver from a seropositive donor, and four of them developed primary HHV8 infection. Two of these patients had lethal nonmalignant illness with systemic involvement and multiorgan failure. Among the 19 HHV8 seropositive recipients, two had viral reactivation after liver transplantation. In addition, an HHV8 seronegative recipient of a seronegative donor developed primary HHV8 infection and multicentric Castleman's disease. In conclusion, primary HHV8 infection transmitted from a seropositive donor to a seronegative liver transplant recipient can cause a severe nonmalignant illness associated with high mortality. Donor screening for HHV8 should be considered in geographic areas with a high prevalence of such infection.

Key words: Donor/recipient matching, donor screening, HHV8, HHV8 seroprevalence, liver transplantation

Abbreviations: CMV, cytomegalovirus; HHV8, human herpesvirus 8; HCC, Hepatocellular carcinoma; HCV, hepatitis C virus; IH, immunohistochemical; LFTs, liver function tests; LT, liver transplantation; LANA-1, latent nuclear antigen 1; MELD, Mayo end-stage liver disease; MCD, multicentric Castleman's disease; NHL, non-Hodgkin's lymphomas; PEL, primary-effusion lymphoma; PCR, polymerase chain reaction; TPHA, Treponema pallidum hemagglutination test.

Received 29 June 2011, revised 04 August 2011 and accepted for publication 14 August 2011

Introduction

Human herpesvirus 8 (HHV8) is a member of the gammaherpesvirus family, and is known to be lymphotropic. It was first described in 1994 by Chang et al. (1), and was originally identified within a distinct subgroup of acquired immunodeficiency viruses related to non-Hodgkin's lymphomas (NHL), playing a pathogenic role in Kaposi's sarcoma (KS), multicentric Castleman's disease (MCD) and primary-effusion lymphoma (PEL). In solid organ and bone marrow transplant recipients, HHV8 infection is associated with the development of malignant disease (KS and MCD). However, primary HHV8 infection and reactivation of infection have also been associated with severe or even fatal nonmalignant complications in immunodepressed patients, in particular after autologous peripheral stem cell transplantation and kidney transplantation (2,3).

The serologic prevalence of HHV8 infection has been studied in different population settings at different levels of risk of infection, and seems to be heterogeneous globally. In fact, the highest percentage of HHV8 seroprevalence has been recorded in healthy blood donor populations, exceeding 50% in sub-Saharan Africa (4). In Italy, the overall prevalence is around 14%, with the highest prevalence recorded in southern Italy (up to 18.3%) (5). It increases throughout childhood and reaches a plateau by adolescence, suggesting that transmission occurs mainly in the community, probably through saliva or other nonsexual routes (6). Moreover, there is strong evidence that HHV8 can be transmitted by blood transfusion, with seroconversion occurring 3–10 weeks after transfusion (7). Viral transmission has been also associated with drug injection and transplantation of infected organs. In kidney graft recipients, an HHV8 seroprevalence of 6.4% pretransplant increased to 17.7% 1 year later, supporting the hypothesis of donor transmitted HHV8 infection (8,9). Few data are available on HHV8 infection in liver transplant

recipients. A study from a transplant center in France showed an HHV8 seroprevalence of 2.4% in liver transplant recipients, and of 3.3% in organ donors (10). In the same study, primary HHV8 infection developed in four HHV8 seronegative recipients of seropositive donors, with disseminated Kaposi's disease and fatal outcome in two of them. More recently, our group reported a case of primary HHV8 infection after liver transplantation (LT), associated with a fatal, nonmalignant, disseminated illness (11).

The aim of this prospective study was to evaluate the prevalence of HHV8 infection in liver transplant recipients and their organ donors, the risk of primary infection and HHV8 reactivation and the clinical impact of HHV8 infection in the posttransplant period.

Material and Methods

Patients

From August 2006 to July 2010, 215 adult HIV-negative liver transplant candidates were transplanted at our multiorgan transplant institute (28 patients underwent living-related LT). The mean age of the transplant recipients was 54 (range: 18–68), and the gender ratio 3.6 (169 men, 46 women). Major indications for transplantation were hepatitis C virus (HCV)-related cirrhosis in 122 patients, hepatitis B virus (HBV)-related cirrhosis in 29 patients, alcohol-related cirrhosis in 17 patients, fulminant hepatic failure in 3 patients and other causes in 44 patients (10 cryptogenic liver disease, 5 polycystic liver disease, 5 Wilson's disease, 5 primary sclerosing cholangitis, 3 primary biliary cirrhosis, 3 liver metastasis from intestinal carcinoid, 3 hemochromatosis, 2 hemangioendothelioma, 1 familial amyloid polyneuropathy, 1 cystic fibrosis, 1 biliary atresia, 1 nonalcoholic steatohepatitis, 1 Budd Chiari syndrome, 1 autoimmune hepatitis, 1 primary hyperoxaluria type 1 and 1 thalasso drepanocytosis). Hepatocellular carcinoma (HCC) was present in 48.8% of the recipients. The mean Mayo End-Stage Liver disease (MELD) score was 22 (range: 6–48). Thirty patients died in the first-year posttransplantation. The overall 1-year survival posttransplantation was 87.1%.

Immunosuppression consisted of basiliximab 2 mg intravenously in the anhepatic phase and at day 4 posttransplantation, and tacrolimus (target levels 8–10 ng/mL during the first month, 6–8 until the sixth month and 4–6 thereafter). We used a steroid bolus (methylprednisolone 500 mg IV) in the anhepatic phase, and prednisone 25 mg/daily from day 1, with progressive tapering and suspension at the end of the first month posttransplantation until April 2008. From May 2008, we no longer administered steroids after LT. The tacrolimus dosage was not modified in the event of recipient/donor mismatching, but was gradually tapered until cessation only in case of HHV8 primary infection associated with systemic involvement.

Cytomegalovirus (CMV) serology (anti-CMV IgG) was checked in all recipients at the time of inclusion on the waiting list, and in all donors at the time of organ procurement. We did not use an antiviral prophylaxis for CMV, but adopted a preemptive therapy with valganciclovir 900 mg per os bid or ganciclovir 5 mg/kg/daily IV only in case of CMV infection (CMV-DNA $\geq$ 100 000 copies/mL).

Patients were followed up regularly in the transplant outpatient clinic, where surveillance of medication compliance, monitoring of liver biochemistry and virologic screening were carried out by our medical and transplant coordinator teams.

In accordance with the study protocol, all liver transplant candidates were screened for HHV8 serology before the transplant. Organ donor HHV8 serology is not included in the donor screening protocol of Italy's national organ procurement organization, but was done at our institute soon after liver procurement (results were available within 72 h of transplantation). All liver transplant recipients underwent HHV8 surveillance posttransplant by means of real-time polymerase chain reaction (PCR) every week for the first 3 months, and then monthly up to 1 year posttransplantation.

Serologic and molecular studies

HHV8 serology was done to detect antibodies against both HHV8 lytic antigen and latent nuclear antigen (LANA) by using, respectively, Advanced Biotechnologies Inc.'s Indirect HHV8 IgG Antibody Enzyme Linked ImmunoSorbent Assay (ELISA, Advanced;the plates are coated with lysates of purified HHV8 particles) and immunofluorescence assay with lymphoblastoid cells expressing LANA Ag (ALI-FAX). The HHV8 viral load was measured with real-time PCR, using the primer specific "latent nuclear antigen ORF73". The assay was done on Light Cycler (FRET technology). We used six standards (Tib Molbiol, Berlin, Germany) for each run, and Tib Molbiol primers and probes.

The hybridization probes consist of two different oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the PCR cycle.

One probe is labeled at the 5′-end with LightCycler-Red 640 and, to avoid extension, modified at the 3′-end with fluorescein. Only after hybridization to the template DNA do the two probes come in proximity, resulting in fluorescein. The donor fluorophore is excited by the light source of the LightCycler Instrument, and part of the excitation energy is transferred to LightCycler-Red 640, the acceptor fluorophore. The LightCycler instrument then measures the emitted fluorescence of the LightCycler-Red 640.

Our results indicate that the assay's lowest limit for sensitivity is 500 copies/mL. The linear measuring range from 2500 to 50 million copies/mL. The viral load was measured using whole blood samples; total DNA was purified by Qiagen protocol. The quality of the extracted DNA was checked each time, and about 100 ng of DNA was amplified. To detect HHV8 DNA, we also used other types of specimens (fluids, tissues and biopsies), changing the extraction methods according to the different samples.

Histopathological and immunohistochemical (IH) studies

Formalin-fixed and paraffin-embedded 3 μm thick sections were obtained from patients 1, 3 and 4. The IH analysis was done first with the mouse monoclonal antibody against LANA-1 HHV8 (Cell Marque, Rocklin, CA, USA, clone 13B10). The Ventana Benchmark autostainer was used for staining (Ventana Medical System Inc., Tucson, AZ, USA). Sections were counterstained with hematoxylin.

Patients 1 and 4 were also tested for the following antibodies: LANA 2 (mouse monoclonal 1:750), ORF4 (mouse monoclonal 1:25), vIFR1 (mouse monoclonal 1:10), ORF50 (rabbit polyclonal 1:1500) and vIL6 (rabbit polyclonal 1:1000) kindly supplied by Dr. Patrick S. Moore of the University of Pittsburgh Cancer Institute, in Pittsburgh, Pennsylvania. This panel of antibodies against HHV8 recognizes different proteins involved in virus replication (12). Citrate antigen recovery by microwave heating was done in all sections. The specimens were treated with the primary antibodies for 1 h at room temperature, and then with the secondary biotinylated antimouse IgG (ABC Elite Kit, Vector Labs, Burlingame, CA, USA) and with the avid-biotin-peroxidase complex (ABC Elite Kit, Vector*)*. Sections were counterstained with hematoxylin.

In situ *hybridization study*

Formalin-fixed and paraffin-embedded 3-μm thick sections were obtained for patients 1 and 3. The sections were treated for 30 min with proteinase

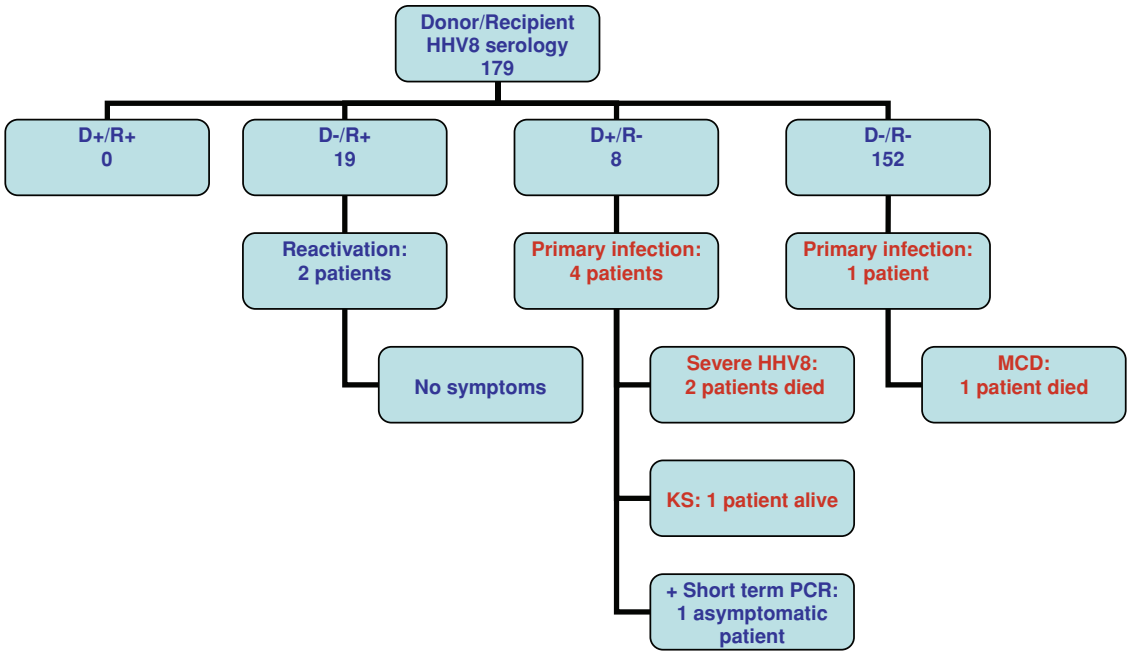

Figure 1: Donor-recipient matching and relevant outcomes according to HHV8 serology in 179 liver transplant cases. MDC = multicentric Castleman's disease; KS = Kaposi's sarcoma.

K in a moist chamber. The tissues were then incubated at 37°C for 2 h with a mix of HHV (type 8) probes (fluorescein labeled; Visionbiosystem, Newcastle upon Tyne, UK). These probes hybridize with a small transcript, designated T1.1 mRNA, that accumulates in the nuclei of infected cells. To detect the hybridization complex, the slides were incubated with an alkaline phosphatase-conjugated Rabbit F (ab') anti-FITC for 30 min. Alkaline phosphatase activity was revealed using NBT-BCIP chromogenic substrate. Positive staining is recognized under the microscope as a dark blue/black color at the site of hybridization.

Results

HHV8 serology was available in all 215 transplanted patients, while, because of logistical problems (i.e. organs procured in other Italian regions or in other countries), HHV8 serology was available in 179 out of the 215 donors (see Figure 1).

The mean age of deceased donors was 54 years (range: 11–93), and of living donors, 30 years (range: 18–54). There were 169 men and 46 women. The cause of death was cerebrovascular disease in 69.07%, trauma in 27.83%, cardiovascular in 2.06% and other in 1.03%. The percentage of donors from other Italian regions, or from abroad, was 9%. Donor serology was positive for anti-HBc in 29 cases (13.4%), for HBsAg in 5 (2.3%) and for anti-HCV in 4 (1.8%). One donor was positive for Treponema Pallidum Hemagglutination Test (TPHA). Four anti-HBc positive donors were found positive for lytic and latent HHV8 antigen. CMV infection occurred in 10 of the 209 transplanted patients (4.8%). The mean time of onset of infection was 47.4 days (27–87). Primary infection occurred in five of the nine seronegative recipients of organs from seropositive donors (56%), whereas an endogenous reactivation occurred in only 5 of the 166 seropositive recipients (3%). The CMV infection was successfully treated according to our protocol (see Section "Material and Methods").

The first 92 patients received steroids after LT, whereas the remaining 123 patients received a completely steroid-free immunosuppressive regimen.

The prevalence of HHV8 (presence of latent and/or lytic antigens) in our adult patients at the time of LT was 10.2% (22 of 215 patients), whereas in the group of organ donors it was 4.4% (8 of 179 donors). Among the assessable 179 donor/recipient pairs, eight seronegative recipients received a liver from an HHV8 seropositive donor.

Several types of serologic assays have been used to measure antibodies to HHV8 and are moderately sensitive and specific for the detection of previous infection with HHV8, but are of limited clinical utility. The sensitivity of serological assays is variable, and ranges from about 80% to greater than 90% (13). In particular, the specificity of our serological assay for the lytic and latent antigens is 89% and 97%, respectively, and this is a possible limitation to attribute to our study.

Among the eight HHV8 seronegative patients who received a seropositive donor, four developed primary HHV8 infection after uneventful LT (average time 117.7 days,

Table 1: Description of patients who developed HHV8 infection after deceased donor LT

PT	Age	Etiology	HHV8 serology D/R	RT-PCR[1] after LT (copies/mL)	Time from LT to infection	TID (days)	Clinical course after HHV8 infection	HHV8-related disease	Outcome
1 ♂	51	HCC+HCV	–/–	8×10^5	158	50	Fever, renal failure, ascites, pleural and pericardial effusion, lymphoadenopathy.	Castleman's disease	Death
2 ♂	58	HCC+HCV	+/–	3.8×10^5	56	96	Fever, renal failure, elevated LFTs, ascites, pleural effusion.	Nonmalignant illness	Death
3 ♂	64	HE	+/–	2×10^4	185	68	Ascites, elevated LFTs, renal failure.	Nonmalignant illness	Death
4 ♀	29	HCC+HCV	+/–	1.5×10^8	224	–	Fever, cutaneous rash, anasarca, lymphoadenopathy.	Kaposi's sarcoma	Alive
5 ♂	60	Alcohol	+/–	6.5×10^3	6	–	None	None	Alive

HCC = hepatocellularcarcinoma; HE = hemangioendothelioma; LFTs = liver function tests.
[1]RT-PCR performed on serum.

range 6–224). Two of them developed a nonmalignant illness characterized by fever, pancytopenia, renal failure, jaundice and high HHV8 viremia; and died of multiorgan failure 68 and 96 days after onset. The third patient developed KS, with complete response to chemotherapy, whereas the fourth patient developed asymptomatic primary infection, and seroconversion after cidofovir treatment (Table 1). The remaining four patients maintained a negative RT-PCR at weekly assessment for the first 3 months after LT, and then at monthly assessment up to 12 months. In all patients who developed HHV8 infection, cidofovir 5 mg/kg every 15 days (1–3 doses) was administered. The dosage was modified in relation to creatinine clearance. Probenecid 4 g in total was administered the same day as the cidofovir injection. In patients who developed KS, liposomal doxorubicin and methilprednisolone were administered.

All patients who developed HHV8 primary infection were pancultured for bacterial, fungi and parasites, and were screened for viral infections (HBV, HIV, CMV, HSV, parvovirus, adenovirus, EBV, HHV8 and HHV6). They were studied with abdominal CT or MRI and lung CT scan with contrast dye. A peripheral blood smear was performed to rule out the presence of blasts. Available fluids were sent to the pathology lab for cytological examination.

Regarding the recipients' characteristics for the four patients who developed HHV8 primary infection, the mean age was 52 (27–64), the blood type was O in three recipients. Three of them had HCC on HCV-related cirrhosis and one had hemangioendothelioma. The mean clinical MELD score at the time of LT was 8 (6–9); only two recipients were transplanted before May 2008, and received steroids post-LT (see section "Material and Methods"). In the four patients who did not develop HHV8 primary infection, the mean age was 62 (60–64). The blood type was uniformly distributed with one recipient for each blood group. Three of them had HCC on HCV-related cirrhosis, and one had cryptogenic cirrhosis. The mean clinical MELD score at the time of LT was 13 (7–26). The four recipients were all transplanted before May 2008, and received steroids post-LT (see section "Material and Methods"). The eight D+/R– recipients were positive for CMV (IgG) before LT, and the gender distribution was similar (3 males:1 female).

Comparing the donors' characteristics, in the group of patients with HHV8- related disease (D+/R–) the mean donor age was 59 (35–83), one of the four donors was anti-HBc positive, and the mean cold ischemia time was 6.25 (4–9 h). While in the D+/R– group free of disease, the mean donor age was 72 (66–87). Three of the four donors were anti-HBc positive, and the mean cold ischemia time was 9.25 (7.3–10 h). The gender distribution and the origin of donors were the same in both groups.

Two recipients among the 19 patients with positive serology before LT had HHV8 virologic reactivation with short-term positive RT-PCR, 4 months (viral load peak less than 2500 copies) and 21 months (viral load peak 9500 copies) after LT, with no clinical signs. Among the 152 seronegative recipients who received an organ from a negative donor, one patient developed MCD 125 days after LT and died of multiorgan failure 50 days later (Figure 1 and Table 1). The rate of systemic HHV8 infection was higher among recipients of grafts from seropositive donors (OR = 151). The seroconversion in mismatched cases for HHV8 (D+/R) occurred in 62.5% of the recipients (5 of 8).

Case reports

For case reports, see Table 2 and Figure 2.

Patient 1

Patient 1 was a 51-year-old man transplanted in October 2006 for alcohol-related cirrhosis. Before LT, the serology was negative for HHV8 and positive for CMV (IgG). The donor was a 56-year-old man who was anti-HBc

Table 2: Immunohistochemical results according to monoclonal antibiodies used in patients 1, 3 and 4

		LANA 1	LANA 2	IL6	ORF50	ORF4	vIRF1
	Samples						
PT 1	(1) Donor liver biopsy	NEG	NEG	NEG	NEG	NEG	NEG
	(2) s/p OLTx liver reperfusion biopsy	NEG	NEG	NEG	NEG	NEG	NEG
	(3) Pleural effusion cytology	POS	POS	NEG	POS	NEG	NEG
	(4) Inguinal lymph node	POS	POS	POS	POS	POS	POS
	(5) Abdominal lymph node	POS	NEG	NEG	NEG	NEG	NEG
PT 3	(1) Donor liver biopsy	POS	–	–	–	–	–
	(2) Ascites citology	POS	–	–	–	–	–
PT 4	(1) Native liver	NEG	NEG	NEG	NEG	NEG	NEG
	(2) s/p OLTx liver reperfusion biopsy	NEG	NEG	NEG	NEG	NEG	NEG
	(3) Cervical lymph node	POS	POS	POS	POS	NEG	NEG

positive. The donor serology for HHV8 (latent and lytic) was negative. Ninety-five days after transplantation, he developed nausea, lack of appetite, low-grade fever, dehydration and moderate renal failure. On physical examination, there were a few small right inguinal lymph nodes and some bilateral axillaries lymph nodes. A few days after admission, he developed ascites, bilateral pleural effusion and mild pericardial effusion. After microbiologic and virologic screening, the blood sample (800 000 copies/mL) and the pleural effusion (1.2 million copies/mL) were found positive for HHV8 DNA. A total body CT scan showed multiple abdominal lymph nodes (maximum 2.8 cm in size), and bilateral inguinal and axillary lymph nodes. The patient was treated with single doses of cidofovir 5 mg/Kg IV, for a total dose of 350 mg, and with probenecid. The tacrolimus was stopped soon after the diagnosis of MCD. We did not use chemotherapy.

The cytologic examination of the pleural liquid showed atypical lymphoid cells (predominantly CD20 positive cells). IH analysis showed lymphocytes positive for HHV8 LANA 1, LANA 2 and ORF50 antigen, and the patient underwent inguinal lymph node excision to rule out lymphoma. The histology showed an atypical reactive lymph node with histological changes suggestive of MCD. Lymphocytes were positive for antibodies LANA 1, LANA 2, IL6, ORF50, ORF4 and vIRF1, all showing a different pattern. In particular, LANA 1-Ab, vIRF1-Ab, vIL6-Ab showed a speckled pattern in a lymphocyte subpopulation of the mantle zone similar to that described by Parravicini et al. (12) Lymphocytes predominantly in the mantle zone were positive for LANA 2-Ab and ORF50-Ab, showing a diffuse nuclear staining pattern, and ORF4-Ab showing a nuclear speckled staining pattern (Figure 3).

A liver biopsy was performed because of increases in liver function tests (LFTs), and showed marked lobular reactivity, with scattered apoptotic hepatocytes, diffuse Kupffer cell hypertrophy and sinusoidal lymphocytic inflammation. The PCR on liver biopsy was positive for HHV8 (1 million copies/µg DNA).

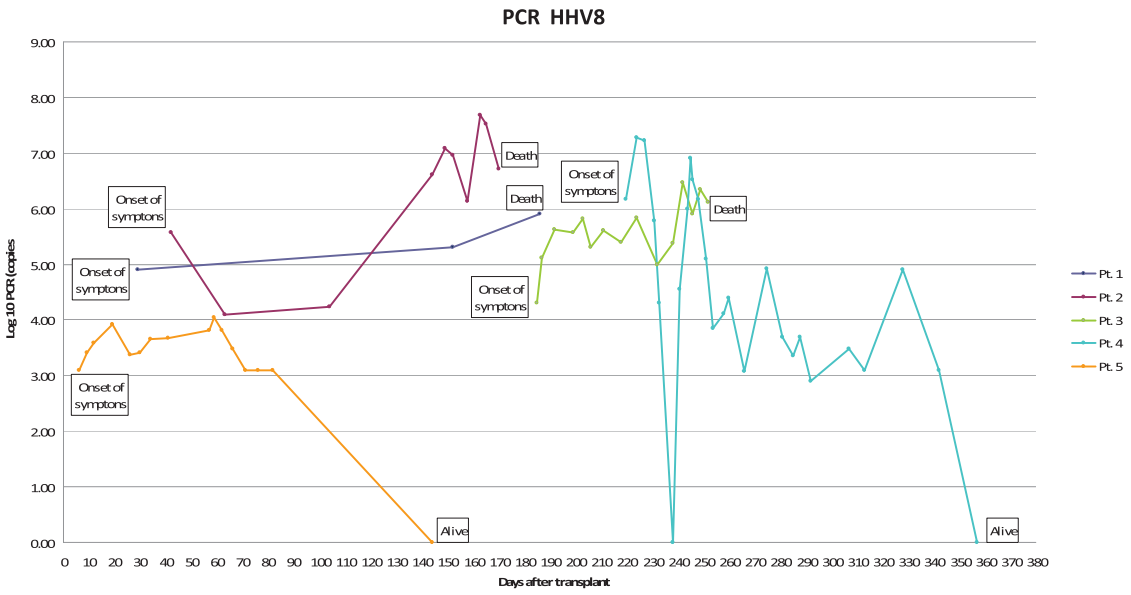

Figure 2: Time-related serum RT-PCR viral load after diagnosis of primary HHV8 infection in five transplanted recipients.

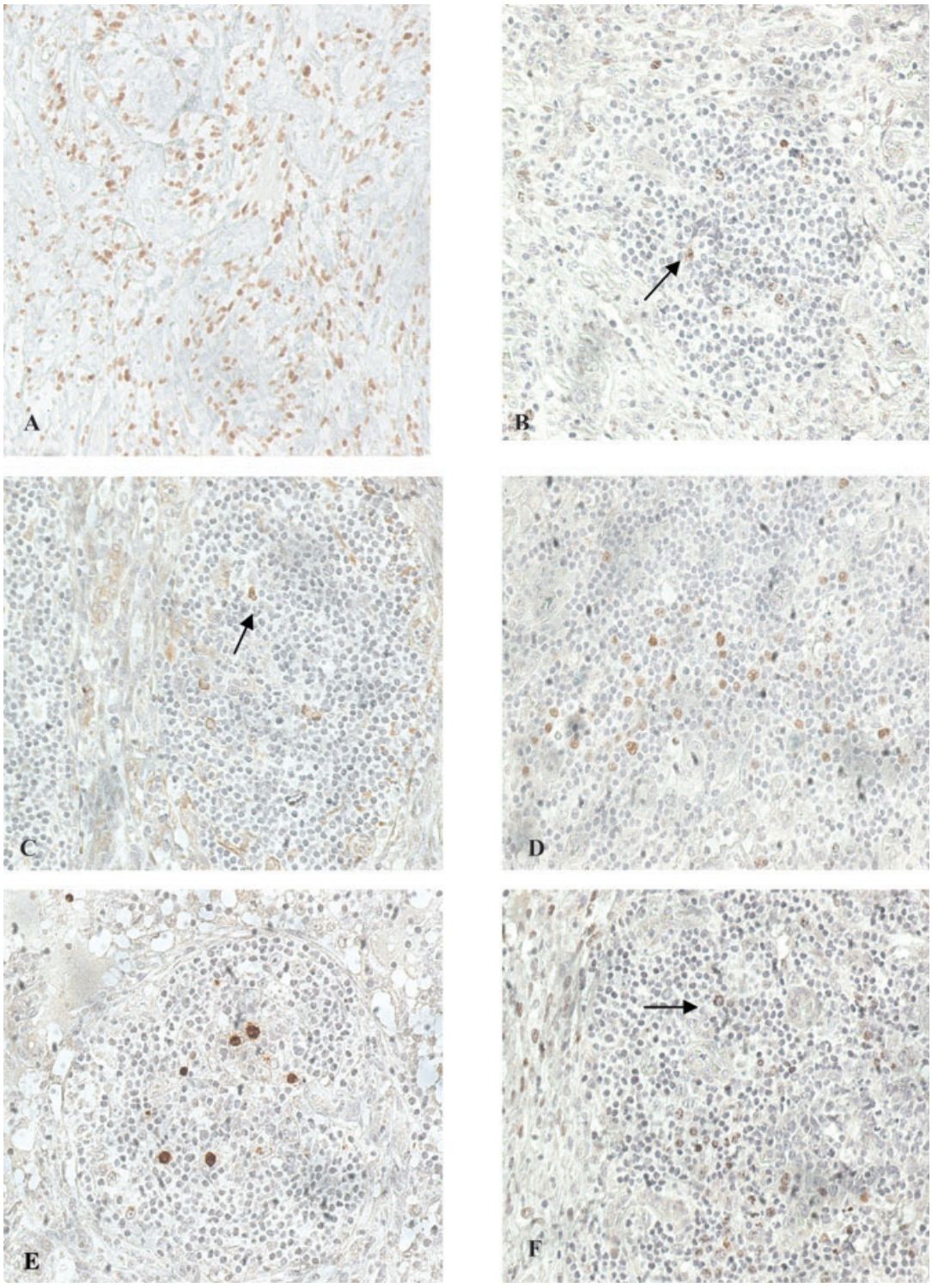

Figure 3: Immunohistochemistry in the inguinal lymph node of patient 1. (A) LANA 1 protein expression is localized in the lymphocytes of the mantle zone, with a nuclear speckled staining pattern. (B) vIRF1 and (C) vIL6 positive cells are localized in the mantle zone. (D) LANA 2, (E) ORF50, (F) ORF4 expression is predominantly in the lymphocytes of the mantle zone, with a nuclear diffuse and speckled pattern, respectively.

The patient had a rapid course, with multiorgan failure, and died in March 2007, 50 days after admission. An autopsy was performed. Histological evaluation of the liver, kidney and lungs samples showed massive necrosis of the parenchyma. Histological examination of intra-abdominal lymph nodes showed viable tissue, with marked depletion of lymphocytes. Few lymphoid aggregates showing positive LANA 1-Ab, negative LANA 2-Ab, IL6-Ab and vIRF1-Ab were recognized. Diffuse background staining with ORF50 and ORF4 antibodies limited the adequacy for interpretation. The HHV8 DNA RT-PCR was positive in lymph nodes (116 000 copies/μg DNA), lungs (5500 copies/μg DNA), liver (675 copies/μg DNA) and kidney tissue (230 copies/μg DNA).

Patient 2

Patient 2 was a 58-year-old man transplanted in September 2007 for HCC on HCV-related cirrhosis. Before LT, the serology was negative for HHV8, and positive for CMV (IgG). The donor was a 69-year-old female. The donor serology for HHV8 was positive. The patient was admitted 56 days after LT for fever and cough. Blood screening found him positive for CMV DNA (RT-PCR > 100 000 copies/mL) and HHV8-DNA (RT-PCR: 380 000 copies/mL). The X-rays showed bilateral pleural effusion. The CMV infection was cleared in 12 days after preemptive treatment with valganciclovir. During hospitalization, he was treated first with ampicillin 2 gm IV daily, followed by levofloxacin 500 gm IV daily and vancomycin 500 mg-1 gm IV daily. The HHV8 DNA in the clinical course increased up to 12 million copies, and the viral infection was treated with three doses of cidofovir 320 mg, and probenecid 1 gm, with general improvement, and HHV8-DNA reduction (RT-PCR: 135 000 copies/mL). The lung CT scan showed moderate bilateral pleural effusion and centimetric epiaortic lymphnodes, with no evidence of pneumonia. The abdominal CT scan was negative for malignancy, and the Doppler ultrasound of the liver was within limits. Thirteen days later, the blood PCR increased to 48 million copies, the pleural fluid was found positive for HHV8 (RT-PCR 34 million copies/mL), and the renal and LFTs rapidly worsened. The peripheral blood smear was negative for blasts. Unfortunately, we could not perform a cytological examination of the pleural effusion to eventually rule out the presence of primary effusion lymphoma. The patient died of multiorgan failure in the intensive care unit 96 days after admission. No autopsy was performed.

Patient 3

Patient 3 was a 64-year-old man transplanted in June 2007 for HCC on HCV-related cirrhosis. Before LT, the serology was negative for HHV8, and positive for CMV (IgG). The donor was an 83-year-old male. The donor HHV8 serology was positive. The postoperative course was complicated by biliary anastomotic stenosis, which was treated with several sessions of ERCP with plastic stent placement. One hundred eighty-five days after LT, the patient was readmitted for ascites and an increase in LFTs. A transjugular liver biopsy showed a pattern of acute cellular rejection and cholestasis associated with cholangitis. The patient was then treated with intravenous steroid pulses, and the tacrolimus dose was increased, with mild improvement of liver enzymes. During hospitalization, he was treated with piperacillin/tazobactam 2.25 g every 8 h, followed by vancomycin 1 g every 12 h, meropenem 1 g every 8 h, and levofloxacin 750 mg daily. The HHV8 PCR was positive, first in the blood (20 000 copies/mL) and, 7 days later, in the ascites (400 000 copies/mL). Because of increasing titers, we decided to treat the patient with cidofovir (a total of three doses). The HCV-RNA level at that time was 1 990 000 UI/mL. The IH staining done on the ascites cytospin (in two different moments) showed some typical lymphocytes positive for HHV8 (LANA antigen) and was negative for malignant cells. A second transjugular liver biopsy was done because of an increase in LFTs. Histology showed marked lobular reactive changes, with diffuse hepatocellular swelling, lobular disarray and several acidophilic necrotic hepatocytes. Few questionable sinusoidal lymphocytes stained positive with LANA 1-Ab. Marked ductular reaction was seen in the portal tracts. No acute cellular rejection was identified. A new session of ERCP was done, with plastic stent replacement. The abdominal MRI showed intrahepatic biliary dilatation and a secondary surrenalic lesion. There was no evidence of lymphoadenopathy on physical examination and abdominal and lung CT scans. Despite the antiviral treatment, the level of HHV8 DNA remained high (2.25 million copies/mL). The patient finally developed renal and liver failure, and died of multiorgan failure 68 days after admission. No autopsy was performed.

Patient 4

Patient 4 was a 29-year-old woman who underwent LT for hemangioendothelioma in September 2008. Before LT, the serology was negative for HHV8, and positive for CMV (IgG). The donor was a 47-year-old man whose HHV8 serology was positive. Six months later, the patient developed fever, weakness, severe sinus tachycardia and maculopapular skin rash. Bacterial cultures and routine viral PCRs were negative. The patient's blood PCR for HHV8-DNA was positive (1 500 000 copies/mL). A total body CT scan showed multiple lymphoadenopathies on the neck, chest and abdomen, but no other abnormalities. She was then treated with cidofovir. On day 5, she had continuous fever (40.3°C), severe sinus tachycardia (160/min), ARDS, oligoanuria, skin rash, severe anemia and severe thrombocytopenia (5 000/mL). Blood pressure, white blood cell count and graft function tests were normal. Her HHV8-viral load was 19 000 000 copies/mL. She then received a second dose of cidofovir. Cervical lymph node biopsy showed histological features of KS. Positive IH stains for HHV8 were seen with antibodies LANA 1, LANA 2, IL6 and ORF50. Liposomal-doxorubicin (20 mg/m^2 q2weeks) and a low dose of steroids (methylprednisolone 0.5 mg/kg bid) were then administered. After 72 h (day 11), the

patient had no fever, and the HHV8 viral load decreased to 600 000 copies/mL. It became undetectable on day 18. The patient had two additional doses of cidofovir, and five additional doses of liposomal-doxorubicin. During the next 18 months of follow-up, the viral load was constantly below 2500 copies/mL, until the 15th month, when it became undetectable. The patient is alive and well.

Patient 5

Patient 5 was a 60-year-old man who was transplanted in May 2010 for HCC on HCV-related cirrhosis. Serology was negative for HHV8 and CMV before LT. The donor was a 35-year-old man whose HHV8 and CMV serology were positive. Six days after LT, the patient was found positive for HHV8-DNA (PCR 2600 copies/mL) on routine screening, with no clinical symptoms. The maximal viral load peak recorded was 11 100 copies/mL. He was treated with two doses of cidofovir (325 mg IV each), with evidence of slow reduction of HHV8-DNA. The patient had a seroconversion 63 days after diagnosis and became negative in the fourth month after LT.

Discussion

We report five HIV-negative liver transplant patients who developed primary HHV8 infection soon after LT. A malignant disease associated with HHV8 infection was found in two of them (KS in one and MCD in the other). In the other two patients, we observed the onset of systemic, rapid course illness, characterized by fever, anemia, jaundice and pancytopenia, leading to multiorgan failure and death. A similar clinical scenario was first described in one bone marrow transplant recipient as viral reactivation, and in one kidney transplant recipient as primary HHV8 infection (2). The last patient was found positive for HHV8-DNA 6 days after LT and did not develop any symptoms. The donor serology was positive for HHV8, though we cannot exclude a blood donor origin of the infection.

In our patients, HHV8 was found in the blood, effusions and, when available, in tissue (e.g. lymphnodes, liver and kidney). These findings confirm, as previously reported (10), that HHV8 primary infection is life-threatening after LT because of immunosuppression, and that available treatments are not effective. To the best of our knowledge, this is the first report on the occurrence of HHV8-associated, nonmalignant systemic illness in liver transplant recipients. Our study confirms that HHV8 primary infection (often donor-derived) is associated with a more severe clinical course and higher mortality compared with HHV8 reactivation in solid organ transplant recipients.

One of our patients developed KS, which is the most common HHV8-related complication posttransplantation. In a recent multicenter study involving four Italian transplant centers, the KS incidence rate was studied among kidney, liver and heart recipients, and was, respectively, 2.5, 0.7 and 4.0 cases per 1000 individuals per year (14). The use of multiple immunosuppressants was found to be an independent risk factor (14,15). Interestingly, the KS that occurs in renal transplant patients is associated principally with pretransplant positive HHV8 serology (with no major impact on survival), meaning that the trigger of the disease is the HHV8 reactivation (10), whereas in liver recipients it occurs with *de novo* HHV8 infection, with an often fatal clinical course (10,16).

HHV8-related MCD is a rare phenomenon in HIV-negative liver transplant recipients. The first patient described died of multisystemic involvement despite a decrease in tacrolimus dosing, whereas the second patient achieved a sustained remission after weaning of immunosuppression and administration of antiviral drugs (17,18). In our series, the patient with MCD showed a more aggressive clinical course, and no response to treatment.

We believe that the HHV8 transmission in D–/R– cases was conceivably related to blood product infusion. Unfortunately, we could not screen all the blood and plasma donors of our liver transplant recipients.

The HHV8 seroprevalence in recipients (10.2%) and donors (4.4%) in our study is lower than previously reported in southern Italy. This may be attributable to recent improvements in social conditions. The discrepancy in seroprevalence among recipients and donors may be related to the different sociodemographic characteristics of the two populations (older age and higher risk of exposure to blood-transmitted infections in the recipients). The risk of HHV8 infection in transplant recipients is correlated with the prevalence of infection in the patient population, and in the organ donor population. In our institute, HHV8 seronegative liver transplant recipients are exposed to a 5% risk (1 patient in 20) of receiving an organ from an HHV8 seropositive donor (eight seropositive donors allocated to a group of 160 seronegative recipients), and of developing a clinically relevant primary infection.

The effect of antiviral agents on HHV8 replication has not been extensively studied, and *in vivo* data are limited. A double-blind, placebo-controlled, crossover trial assessed the efficacy of oral valganciclovir (900 mg once daily) versus placebo in 26 men infected with HHV8 (19). Valganciclovir use was associated with significantly less oropharyngeal shedding of HHV8 as detected by daily quantitative PCR assays (23% vs. 44% with placebo). In contrast, a report of seven HIV-infected individuals receiving intravenous ganciclovir or foscarnet for CMV retinitis found no difference in pre- and posttreatment HHV8 DNA levels in peripheral blood mononuclear cells (20). Cidofovir, as well as other antiviral agents, has been found to have *in vitro* ability to block stimulation of lytic replication in HHV8 infected cell lines (21).

In light of our results, we believe that HHV8 screening should be part of the serological workup in patients who

undergo LT, at least in endemic areas. In the future, we will continue to accept mismatched cases because of donor scarcity, and though ideally it would be useful to screen blood products for seronegative pairs, the cost-benefit balance would not justify such a strategy. Donor status can be measured by HHV8 serology and PCR of stored blood after LT, allowing for early identification of a seronegative recipient transplanted with a seropositive organ and a reduction of the burden of immunosuppression, at least in the initial period (e.g. the first 6 months). However, no effective antiviral treatment has been identified, and the role of posttransplant surveillance of HHV8 viremia has yet to be satisfactorily defined.

Acknowledgments

We thank Dr. Patrick S. Moore (University of Pittsburgh Cancer Institute, Pittsburgh, PA) for part of the monoclonal antibodies used to detect HHV8 in this study, and Warren Blumberg for his help in editing this paper.

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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