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Primary herpes simplex virus type-2 infection as a cause of liver failure after liver transplantation

Key words:

HSV; transplantation; liver failure

Abstract: We report a case of fatal primary herpes simplex virus type-2 (HSV-2) infection following liver transplantation, which manifested with fever and liver failure in the absence of mucocutaneous disease. The infection was characterized by high levels of HSV DNA in blood and the patient's inability to mount HSV-specific T-cell responses while showing preserved T-cell responses against cytomegalovirus. The donor was HSV-1 immunoglobulin G (IgG) seronegative and HSV-2 IgG seropositive, whereas the recipient was HSV-1 and HSV-2 IgG seronegative, suggesting that the graft may have been the source of the infection. In HSV-seronegative recipients of grafts from HSV-seropositive donors, HSV infection should be included in the differential diagnosis of a febrile illness, regardless of the absence of mucocutaneous disease. In this setting, real-time polymerase chain reaction applied to blood samples provides a sensitive, rapid, and quantitative diagnostic tool.

In recipients of solid organ transplantation, herpes simplex virus (HSV) infection may result in visceral dissemination with multiorgan involvement, including fulminant hepatitis. The infection most commonly results from reactivation of latent virus in persons who are HSV seropositive at the time of transplantation. Antiviral prophylaxis is effective in preventing recurrent HSV disease in these patients (1). Severe disease following primary HSV infection has rarely been described. A few prospective studies failed to detect HSV disease among HSV-seronegative renal and cardiac transplant recipients (2, 3). In one other study, only 1 of 26 HSV-seronegative liver transplant recipients developed HSV disease post transplant (4).

Transmission of viruses through grafted organs is an increasingly recognized problem, with recent examples in-

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cluding West Nile virus and rabies. There are no studies addressing the incidence of HSV infection after transplantation of organs from HSV-seropositive donors to HSV-seronegative recipients. However, case reports have suggested HSV transmission through transplantation in recipients of renal, pancreas, and heart grafts (5–8).

Case report

A 44-year-old woman with intrahepatic epithelioid hemangio-endothelioma underwent elective orthotopic liver transplantation following chemotherapy with doxorubicin. Pre-transplant serology showed no evidence of infection with hepatitis B, hepatitis C, or human immunodeficiency virus. Specific immunoglobulin G (IgG) was detected against cytomegalovirus (CMV), Epstein-Barr virus (EBV), and varicella zoster virus (VZV), but not against HSV.

The transplant procedure was uneventful. Two days later, immunosuppressive therapy was started with tacrolimus (3 mg twice daily). On day 6, a routine liver biopsy demonstrated moderate cellular rejection. Laboratory results showed a moderate elevation of aspartate and alanine aminotransferases (AST 134 U/L; ALT 171 U/L), γ -glutamyl transpeptidase (γ GT 537 U/L), and bilirubin 83 μ M/L. She was given methylprednisolone (1 g once daily) for 3 days.

On day 10 she became febrile (38.1°C). Abnormal laboratory findings included AST 312 U/L, ALT 251 U/L, γ GT 720 U/L, bilirubin 21 μ M/L, C-reactive protein 156 mg/dL, hemoglobin 6.6 g/dL, white blood cells 12×10^9 /L, neutrophils 11.2×10^9 /L, and lymphocytes 0.63×10^9 /L. Empiric antibiotic therapy with piptazocin was started. Routine microbiological cultures of blood, urine, and stool were negative. On day 11 methylprednisolone (1 g once daily for 3 days) was given for a suspected second episode of acute liver rejection.

The clinical condition worsened. The antimicrobial therapy was changed to liposomal amphotericin, teicoplanin, and meropenem. Laboratory tests showed a further deterioration of the liver function with AST 5157 U/L, ALT 1979 U/L, γ GT 1030 U/L, bilirubin 27 μ M/L, international normalized ratio 5.2, prothrombin time 52 s, and activated partial thromboplastin time 73 s. On day 15, HSV-2 DNA (15.9 \log_{10} copies/mL) was detected in whole blood by real-time polymerase chain reaction (PCR) assay. Blood PCR for

CMV, EBV, and adenovirus was negative. On the same day, a liver biopsy showed extensive necrosis with ground-glass intranuclear inclusions in the surviving hepatocytes, with positive intranuclear staining for HSV (Fig. 1). Immunostaining for CMV, EBV, and adenovirus was negative. Intravenous acyclovir (10 mg/kg 3 times daily) was started. The patient continued to deteriorate. On day 16 she required transfer to intensive care, where she died one day later with multiorgan failure. The autopsy findings confirmed widespread confluent hepatic necrosis and multiorgan failure as the cause of death. Immunostaining detected HSV in the liver, hepatic nerve endings (Fig. 1), adrenal glands, and macrophages of spleen and lymph nodes, but not in the lungs, stomach, rectum, myocardium, pericardium, kidneys, or cervix.

By retrospective testing of stored blood samples, the HSV PCR was found to be negative (limit of detection 100 copies/mL) on the day of transplant (Fig. 2). HSV-2 DNA was first detected in blood on day 6 post transplant and remained detectable at high level until the time of death. Retrospective HSV immunostaining of the liver biopsy taken on day 6 was negative (not shown). Retrospective HSV type-specific serology (HerpeSelect HSV-2 ELISA IgG, Focus Diagnostics, Cypress, California USA) showed that the patient was HSV-

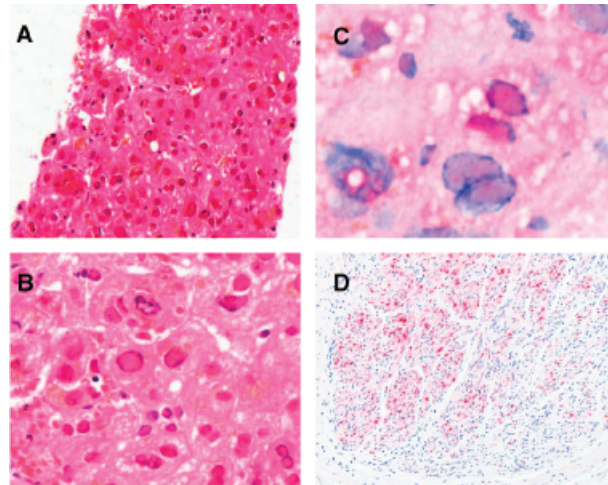


Fig. 1. (A) Liver biopsy. Zonal necrosis is evident and the hepatocytes at edge of the necrotic area show pale nuclei (hematoxylin and eosin [H&E] $\times 200$). (B) Liver biopsy. Higher magnification shows in detail the nuclear changes of the hepatocytes (H&E $\times 400$). (C) Liver biopsy. Immunohistochemistry with anti-herpes simplex virus (HSV) antibodies shows nuclear staining of hepatocytes ($\times 100$). (D) Autopsy: anastomotic nerve. Immunohistochemistry with anti-HSV antibodies shows staining of peripheral nerve from the liver anastomosis ($\times 100$).

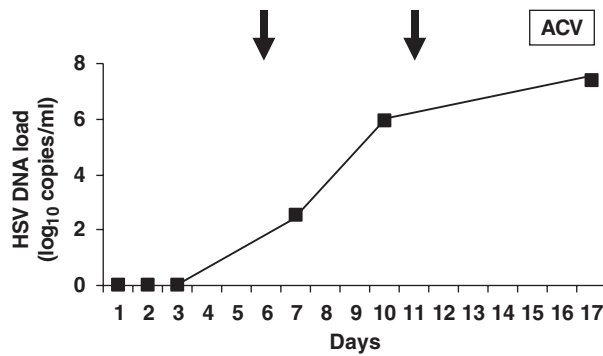


Fig. 2. Kinetics of herpes simplex virus (HSV) type-2 viraemia. The patient was on immunosuppressive therapy with tacrolimus and received two 3-day courses of methylprednisolone (1 g/day) at day 6 and at day 11 (arrows). Intravenous acyclovir (ACV) (10 mg/kg 3 times daily) was initiated at day 15.

1 and HSV-2 seronegative before transplant, on the day of transplant, and at the time of death.

To assess T-cell function, stored peripheral blood mononuclear cells collected at multiple time points after transplant were stimulated *in vitro* with either HSV-2 or CMV lysates to measure T-cell-mediated production of IFN- γ by ELISPOT assay. A good T-cell response was measured against CMV, whereas no HSV-specific T-cell responses were detected throughout the post-transplant period (Fig. 3).

The donor was an unrelated adult female who at the time of transplantation was HSV, CMV, EBV, and VZV IgG seropositive. By retrospective testing, HSV-2 type-specific IgG was detected in serum collected before transplant, whereas HSV-1 type-specific IgG was negative. The HSV-2 IgG antibodies showed a high avidity index (54%) by a modified HerpeSelect HSV-2 ELISA, which was consistent with an established rather than recent infection (9). No HSV-2 DNA was detected in plasma by PCR. Furthermore, HSV-2 was not detected by either immunostaining or PCR in the liver biopsy collected at the time of explant.

Discussion

The case reported was HSV-1 and HSV-2 seronegative pre-transplant and acquired a primary HSV-2 infection around the time of transplantation. Although HSV viraemia was already present on day 6, the infection was not detected until day 15. During this interval, 2 courses of methyl-

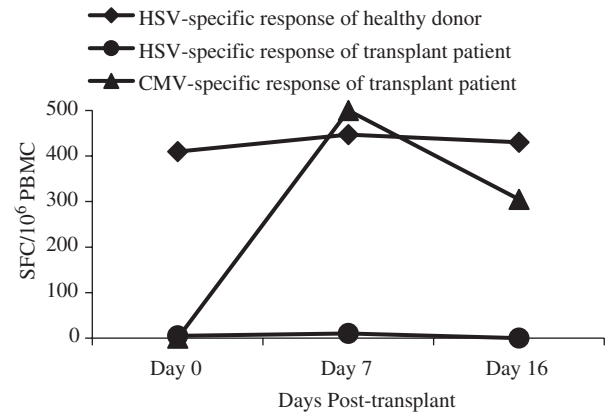


Fig. 3. T-cell interferon (IFN)- γ response measured by ELISPOT. Peripheral blood mononuclear cells (PBMC) were stimulated *in vitro* with either herpes simplex virus type 2 (HSV-2) lysates at 10 μ g/mL or cytomegalovirus (CMV) virus lysate (strain AD169, ABI, MD, USA) at 5 μ g/mL. Results obtained with PBMC collected from the transplant recipient at 3 time points after liver transplantation are shown. HSV-specific responses measured with PBMC from a healthy donor served as a positive control. Results are reported as mean (duplicate wells) number of spot-forming cells (SFC) per 10⁶ PBMC as measured by an automated reader. Responses to mitogenic stimulation with PHA was 1161 SFC/10⁶ PBMC, whereas responses to the negative control tissue culture media were <5 SFC/10⁶ PBMC.

prednisolone were administered, including one given to treat biopsy-proven rejection that coincided with the onset of viraemia. The patient did not develop HSV-2-specific antibodies during the 17 days of follow-up. However, in newly acquired infections, development of HSV-type-specific antibodies occurs at median 21 days after presentation (10). T-cell-mediated immune responses are critical for HSV containment. Whereas the patient's T-cell response against CMV was intact post transplant, no response was elicited *in vitro* against HSV antigens. Thus, lack of preexisting HSV immunity, ongoing immunosuppressive therapy, and impaired ability to mount a primary T-cell immune response against HSV contributed to the overwhelming infection in this case.

The route of transmission remained unclear. In two previously published case reports (6, 7), severe HSV-2 infection developed after renal transplantation and the viruses detected in different transplant recipients showed identical restriction endonuclease polymorphism patterns, supporting a common source for the infection through the graft. In this case, the detection of HSV-2 antibodies in the donor indicates that the infection may have occurred through the transplant, although other sources cannot be excluded

with certainty in the absence of a direct comparison of the 2 virus strains. We were unable to obtain virus from the donor for such a comparison.

The donor had an established HSV-2 infection. HSV-2-seropositive persons periodically reactivate the infection, shed virus from multiple anatomical sites, and are potentially infectious. It could be postulated that the donor's blood or liver served as the vehicle for HSV-2 transmission, although there are no data to indicate whether recurrent HSV-2 infection is associated with viraemia or with intrahepatic virus replication. HSV-2 DNA was not detected in the donor liver biopsy taken at the time of the explant and the donor was not viraemic at that time. However, as only plasma was available for retrospective HSV PCR testing, it is possible that the virus was harbored in the donor's blood lymphocytes and monocytes (11). Other potential sources of the infection include vascular endothelial and smooth muscle cells, nervous tissue, and tissue macrophages (12, 13).

Antiviral drugs used for prophylaxis of CMV infection are also effective in preventing HSV disease. Recent guidelines recommend that when preemptive anti-CMV regimens are used, the issue of HSV prophylaxis should again be addressed (14). In the UK, however, prophylaxis against HSV is not routinely recommended for solid organ transplant recipients. This reflects the lack of evidence supporting its use, especially in the context of HSV-seronegative recipients (15).

This case illustrates the importance of considering HSV infection in HSV-seronegative transplant recipients who develop a febrile illness following transplantation, regardless of the absence of muco-cutaneous disease. Real-time PCR to demonstrate HSV viraemia would have detected the infection at a much earlier stage in this case, allowing the prompt initiation of antiviral therapy and the avoidance of a second course of steroid therapy on day 11. Because of declining rates of HSV-1 acquisition in childhood, the number of HSV-seronegative transplant recipients at risk for primary infection is likely to be increasing (16). The overall risk of disease and the role of antiviral prophylaxis remain to be determined in prospective studies.

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