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Case report

Donor-derived human herpes virus 8-related Kaposi's sarcoma in renal allograft ureter

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Abstract: We present a case of human herpes virus 8 (HHV8)-associated Kaposi sarcoma (KS) occurring in a renal allograft ureter from a male donor. The female patient presented with a rising creatinine due to ureteric obstruction, and subsequent histological examination of the excised tumor revealed a KS. The tumor tested positive for HHV8 antigen and, using *in situ* hybridization to identify X and Y chromosomes, we were able to demonstrate that the tumor was of male origin. In the absence of any other KS lesions, this suggested that the tumor arose due to reactivation of latent HHV8 in the donor tissue, permitted by the recipient's immunosuppression. The patient was managed by a gradual reduction in immunosuppression and there has been no subsequent recurrence of the tumor. KS in renal transplantation is discussed in detail including the possible utility of pre-transplant HHV8 screening.

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Kaposi's sarcoma (KS) was first described by Moritz Kaposi, a Viennese dermatologist, in 1872. The blue–purple lesions were seen in elderly men and thought to be slow-growing neoplasms. KS usually arises in the skin as plaques or nodules occurring most frequently in the patients' legs, although it can occur at any site. KS is a tumor of mixed fibroblastic and vascular origin and is now recognized to affect 4 distinct patient groups. Classical KS is seen in elderly east European and Mediterranean men, and has a good prognosis. Endemic KS is seen in sub-Saharan Africa and can be aggressive in children. Epidemic KS occurs in patients with human immunodeficiency virus (HIV) infection and is an acquired immunodeficiency syndrome (AIDS)-defining illness. The final group are immunosuppressed patients with solid organ transplants. KS in renal transplant (RT) patients is mainly seen in the skin, but other viscera are also affected, e.g., lung, liver, gastrointestinal tract, and lymph nodes. It is, however, extremely rare for it to affect a transplanted organ. Human herpes derived virus 8 (HHV8) is the etiological agent for KS, and RT patients may develop KS due to reactivated or acquired HHV8 infection. There have been 4 previously reported cases of KS affecting the transplant urothelium (1–4). However, this is the first case, to our knowledge, where the transmission of HHV8 from the donor has led to KS in the recipient allograft.

Case report

A 55-year-old female with end-stage renal failure received a live unrelated RT in India from a male donor. Her original diagnosis was thought to be glomerulonephritis and she was commenced on an immunosuppression regimen of cyclosporin A (CyA), prednisolone, and mycophenolate mofetil (MMF). There were no immediate complications from transplantation and she reached a stable creatinine of 89 μmol/L.

Twelve months after her RT, the patient was admitted with an increasing creatinine level, rising to 157 μmol/L over the course of 1 month. There was no evidence to suggest rejection. Computed tomography scan of the abdomen and pelvis revealed an edematous bulky transplant kidney. Renal ultrasonography demonstrated a dilated collecting system, and a MAG3 renogram confirmed mild

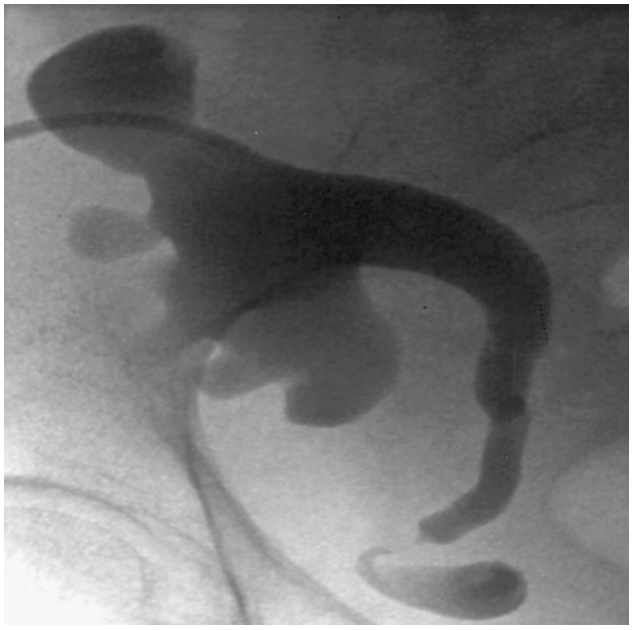

Fig. 1. Nephrostogram showing dilatation of the pelvicalyceal system of the transplant kidney due to a stricture in the distal ureter.

obstruction. A nephrostomy tube was placed and a nephrostogram demonstrated 2 tight strictures at the lower end of the transplant ureter (Fig. 1). Owing to the length of the strictures, open excision and reconstruction was preferred to dilatation of the stricture. A laparotomy was performed. The strictured transplant ureter was found to be hard and nodular. The abnormal distal ureter and a cuff of bladder were excised. Proximally the ureter appeared normal. A re-do ureteroneocystostomy was performed, leaving a stent temporarily to protect the anastomosis.

Histological examination of the tumor showed a spindle cell mass adjacent to the ureteric lumen (Fig. 2A and B). The spindle cells expressed CD34 and HHV8 antigen, consistent with a diagnosis of KS (Fig. 2C and D). DNA extracted from paraffin-embedded tissue was also analyzed for the presence of HHV8 DNA using a polymerase chain reaction (PCR) technique described elsewhere (5). This test gave a strongly positive result consistent with the histological finding of KS. There was no clinical evidence of any other KS lesion in the patient.

Sections of the excised tumor were analyzed for the presence of human X and Y chromosomes using a combined chromosome X and Y satellite probe (MP Biomedicals, Illkrich, France) using *in situ* hybridization (Fig. 3). In the FISH probe for X and Y chromosomes, tissue sections were investigated for the presence of human X and Y chromosomes. Sections were dewaxed and rehydrated in graded alcohols to water. The sections were then treated with sodium thiocyanate to remove excess protein and then permeablized with pepsin. This digestion was stopped with glycine before fixation in paraformaldehyde, washing and dehydrating. Probes were then applied to the slides, which were cover slipped, sealed, and denatured at 80°C before hybridization overnight at 37°C. The following day cover slips were removed and the sections underwent stringency washes in 0.5X standard sodium citrate buffer, washed in phosphate-buffered saline, and mounted in Vectashield Hardset (Vector Labs, Peterborough, England). Slides were then examined using an Olympus fluorescent microscope equipped with Smartcapture software. Human X chromosome is a green (FITC channel) spot; human Y chromosome is seen as a red spot (Cy3 channel). Two green signals indicate a female cell, whereas a male cell shows 1 red and 1 green signal.

The urothelium, as expected, showed all male cells. The deeper tumor tissue showed a mixture of male and female cells with the majority of cells being male strongly suggesting that the tumor arose from the male donor tissue. A num-

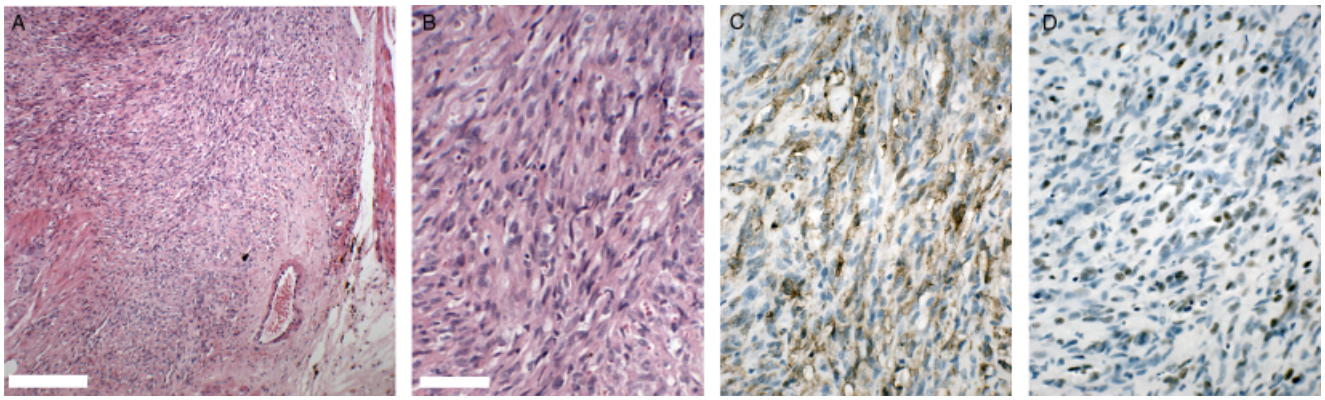

Fig. 2. (A) Low-power photomicrograph of a hematoxylin and eosin (HE)-stained section of the tumor (scale bar 500 μm). High-power photomicrograph of (B) an HE-stained section, (C) a section showing immunohistochemical staining for CD34, and (D) a section showing immunohistochemical staining for HHV8 (scale bar 100 μm).

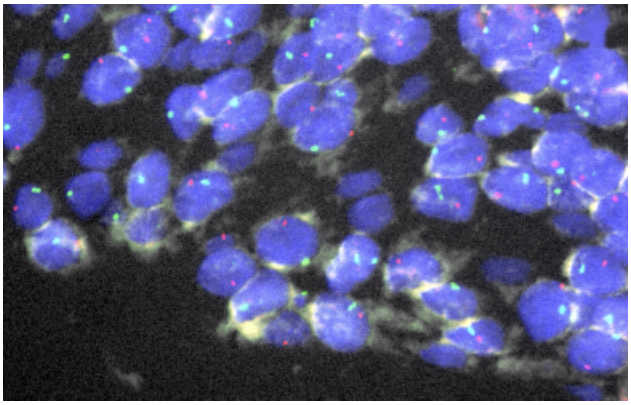

Fig. 3. FISH probe for X and Y chromosomes: Tissue sections were investigated for the presence of human X and Y chromosomes using *in situ* hybridization. The urothelium showed all male cells as expected. The deeper tissue showed a mixture of male and female cells with the majority of cells being male. A number of smaller cells were of female origin suggesting that they were derived from circulating recipient lymphocytes.

ber of smaller round cells were of female origin suggesting that they were derived from circulating lymphocytes.

Attempts were made to combine immunohistochemistry for CD34 or HHV8 with the human X/Y probe to determine if cells were of donor or recipient origin but this was technically not possible. However, areas of cells that were positive for either Kaposi marker contained cells that were of donor origin.

In response to the diagnosis of KS, immunosuppression was reduced through the discontinuation of MMF and stepwise reduction of CyA. This was done with careful monitoring of the patient's CD8, CD19, CD69, and NK T-cell counts (Fig. 4), as has previously been reported for post-transplant lymphoproliferative disorder (6).

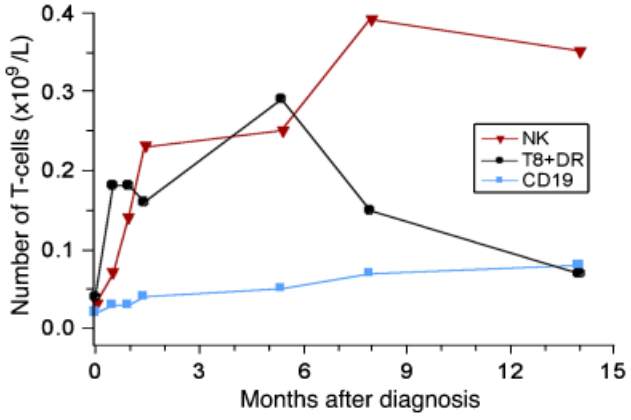

Fig. 4. Graph displaying recovery of immune function during the stepwise reduction in immunosuppression. NK, natural killer cells; T8 + DR, cytotoxic/suppressor T cells; CD19, B lymphocyte surface antigen, T69–CD69 (a marker of T-cell activation).

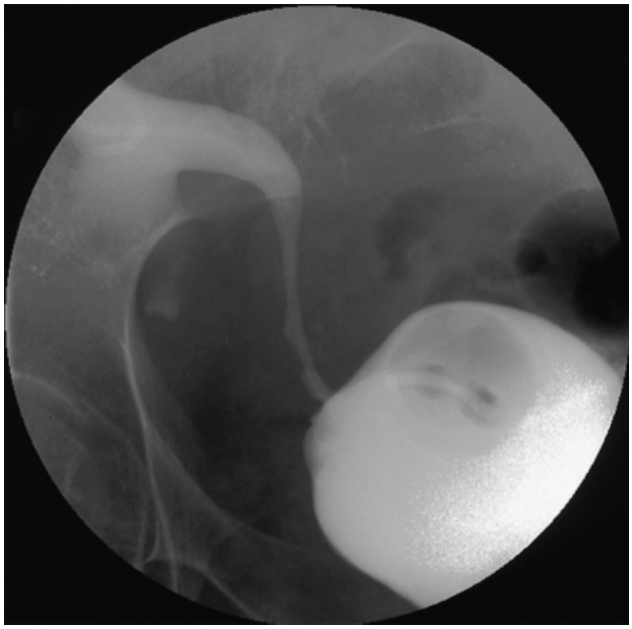

Fig. 5. Cystogram showing rapid reflux of contrast into the transplant ureter with no evidence of further KS lesions or strictures. Biopsies of the ureter confirmed the absence of any residual KS.

Endoscopic follow-up was undertaken at 3 and 6 months. Biopsies and cystography (Fig. 5) showed no recurrence of tumor or stricture. The stent was exchanged at 3 months and removed after 6 months. The patient remains well at 6 years with a functioning graft and a creatinine of 95 μmol/L on 5 mg prednisolone once daily and 50 mg CyA twice daily, with a CyA trough level of 102 ng/mL.

Discussion

Once considered a rare tumor, KS has increased in notoriety due to its association with AIDS and immunosuppression. Indeed, HIV-associated KS is the most common cause of cancer death in men and women in sub-Saharan Africa. The prevalence of KS in the RT population has been reported to occur in 0%–11.8% of cases depending on geographical location. Higher rates are seen in North African and Mediterranean groups compared with more developed countries such as the UK or Australia (see Table 1) (7–29). While we believe the above case was caused by transmission of infected tissue from donor to recipient, HHV8 prevalence is low in India (30) and there has been only 1 Indian report of KS arising in a transplant recipient (31).

KS in transplant patients is associated with HHV8 infection, whether acquired before or at transplantation, and with the use of polyclonal anti-lymphocyte sera. HHV8 or KS-associated herpes virus (KSHV) is a member of the

Geographical distribution of Kaposi's sarcoma (KS) based on published series of renal transplants

Location	Author (reference) year	No. of KS cases	Total	% incidence
Belgium	Sheldon et al. (7) 2000	0	210	0
Spain – Valencia	Martinez Jabaloyas et al. (8) 1994	2	619	0.03
Australia	Sheil et al. (9) 1987	7	4241	0.17
UK – London	Webb et al. (10) 1997	4	1304	0.31
France – Paris 1993	Farge (11) 1993	28	6229	0.45
Hungary	Vegso et al. (12) 2007	12	2535	0.47
France – Lyon	Touraine et al. (13) 1996	12	2500	0.48
South Africa	Margolius et al. (14) 1994	5	989	0.50
Spain – Madrid	Lessan-Pezeshki et al. (15) 2001	3	609	0.50
Canada	Shepherd et al. (16) 1997	7	1300	0.54
Germany	Behrend et al. (17) 1997	1	1497	0.70
Iran	Einollahi et al. (18) 2001	13	1750	0.70
Pakistan	Askari et al. (19) 1999	6	650	0.90
Turkey	Arican et al. (20) 2001	10	954	1.0
Kuwait	Al Mousawi et al. (21) 2001	9	800	1.1
Egypt	Bakr et al. (22) 1997	11	950	1.2
Italy – Milan	Guz et al. (23) 2000	13	854	1.5
France – Paris 2000	Frances et al. (24) 2000	9	400	2.3
Israel	Lal et al. (25) 1998	8	330	2.4
Turkey-Pediatric	Haberal et al. (26) 2000	2	56	3.6
Italy – Rome	Cattani et al. (27) 2000	7	175	4.0
Saudi Arabia	Qunibi et al. (28) 1993	26	630	4.1
Nigeria	Abdu et al. (29) 2005	2	17	11.8

Table 1

gamma herpes virus group, which are associated with malignancies (e.g., Epstein–Barr virus). HHV8 was first identified in AIDS patients with KS and the association is now well established (32). HHV8 DNA has been found in almost all KS lesions examined (5) and the majority, if not all, RT patients with KS have HHV8 antibodies present in their sera (33). Most HHV8 infections are asymptomatic, and in a healthy population of blood donors the prevalence was found to be 2% (34). In a series from France, the seropositivity rate among 400 prospective RT patients was reported higher at 8%. Of those patients, 28% went on to develop KS compared with no cases in the seronegative group, leading the authors to conclude that RT recipients, especially from high-risk groups, should be screened for HHV8 (35). While it is thought that most RT patients who develop KS do so as a result of reactivation of latent HHV8 virus (36), donor–recipient transmission of virus leading to KS has also been demonstrated (37). This group suggested that there might be a need for screening of donors from high-risk groups.

KS presents on average 21 months after transplantation, with a large proportion of tumors (46%) presenting within 12 months (38). Histological diagnosis can be complemented by PCR to confirm the presence of HHV8 DNA in the specimen. The development of KS in RT patients is thought to be attributed to the immunosuppressive agents, cyclosporine in particular, which inhibit the normal mechanisms of tumor immuno-surveillance that are reliant on T cells (39). Initial presentation is often with multicentric outcroppings without metastases. Patients are initially treated by the gradual reduction in their immunosuppression. One group had a 100% success rate, albeit with graft loss of 20%, thought to be a consequence of too rapid reduction in immunosuppression (40). Patients who are put at risk by disseminated disease can be treated with liposomal doxorubicin or radiotherapy. The role of antiviral agents to treat HHV8 infection in the context of KS is unclear, although various agents have been used (41). Recent experience with the proliferation signal inhibitors (PSIs) sirolimus and everolimus suggests that the tumor may

regress without the risk of acute rejection, perhaps because PSIs inhibit angiogenesis through inhibition of vascular endothelial growth factor expression and signaling (42). A recent report recorded KS regression in 11 of 12 cases without allograft rejection (43).

HHV8 screening of transplant donors and recipients is a matter of ongoing debate (44). However there is growing support for screening of high-risk RT recipients. Studies into the HHV8 status of donor–recipient pairs and subsequent KS formation are needed to address this issue.

This case is remarkable because KS mainly affects patients' native tissues, such as the skin, and not the allograft. The location of the tumor and the FISH evidence demonstrating X and Y chromosomes in the tumor strongly suggest that the tumor arose in the male donor tissue. Furthermore, there were no other KS lesions in the patient. Thus, it is highly likely that the HHV8 was reactivated from within the donor tissue, permitted by the recipient's immunosuppression, directly leading to KS formation. Fortunately, this patient has recovered well and has kept the transplant due to a combination of complete surgical excision of the KS tumor and careful immunosuppression reduction.

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