# HHV-8 infection and post-transplant malignancies

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Human herpesvirus 8 (HHV-8)/Kaposi sarcoma associated herpesvirus (KSHV) has been identified in 1994 from the Kaposi sarcoma (KS) tissues of patients with acquired immunodeficiency syndrome (AIDS). HHV-8 has been classified as a gamma-herpesvirus, related to Epstein-Barr virus (EBV) and herpesvirus saimiri. Like other herpesviruses, HHV-8 is a large double-stranded DNA virus that replicates in the nucleus as a closed circular episome during latency but linearizes during virion packaging and replication. HHV-8 viral genome is unique, compared with the genome of other human herpesviruses, as it contains genes associated either with the latent or the lytic phase of the viral cycle, which are homologous to cellular genes, involved in the control of cell cycle and apoptosis. HHV-8 may infect B lymphocytes *in vitro*, but, unlike EBV, is not capable to induce their immortalization. Conversely, HHV-8 is capable to immortalize endothelial cells *in vitro*, in the presence of vascular endothelial growth factor. Microvascular endothelial cells represent a mixed population of lymphatic endothelial cells (LECs) and of blood vascular endothelial cells (BECs), which are both susceptible to HHV-8 infection, although with different efficiencies, being HHV-8 replication higher in LECs. The gene expression profile of KS neoplastic cells, as determined by microarray analysis, is closely related to that of LECs , A comparison of the gene expression micro-array profiles of HHV-8 infected and uninfected LECs and BECs have shown that HHV-8 induces a transcriptional drift of both infected LECs and BECs to move away from the uninfected populations and toward each other. These *in vitro* studies may explain why KS neoplastic spindle cells express not only LEC markers but also BEC markers, *in vivo*. In certain culture conditions, the lymphatic reprogramming induced by HHV-8 infection in BECs is characterized by the down-regulation of blood vascular genes and the induction of about 70% of the main lymphatic lineage-specific genes, including PROX1, a master gene that controls the embryonic development of the mammalian lymphatic system. Among the lymphangiogenic molecules which are up-regulated in both LECs and BECs infected with HHV-8 *in vitro*, angiopoietin-2 is of particular importance, being its expression detectable in KS lesions *in vivo*, and its plasma levels significantly higher in patients with AIDS-KS than in healthy controls. $\alpha3\beta1$ integrin (CD49c/29) has been recognized as one of the *in vitro* cellular receptors involved in the infectious process by the virus. It is interesting to note that *in vivo*, $\alpha3\beta1$ integrin is abundantly expressed in endothelial cells, B cells, monocytes, and epithelial cells, the same target cells in which HHV-8 DNA and HHV-8 transcripts have been detected.

Kaposi' sarcoma is a vascular tumor characterized by thin-walled neovascular formations and proliferating spindle cells. The development of KS in all its clinical forms (classic, AIDS-related, endemic and post-transplant) is invariably associated with infection with HHV8, which is detectable in KS neoplastic cells (endothelial and spindle cells). Post-transplant KS usually appears rather early, after transplantation, being the median interval from organ transplantation to KS of 29 to 31 months (range 3 to 124 months). Ninety percent of transplant patients with KS have cutaneous or mucosal lesions or both types. Visceral involvement occurs in 25 to 30% of patients with kidney transplants and in 50% of those with heart or liver transplants, while purely visceral disease occurs in 10% of patients. The course of KS depends on the level of immunosuppression; the lesions may regress on discontinuation of immunosuppressive therapy, although with a high risk of allograft loss, but may recur after reintroduction of immunosuppressive treatment. The estimated survival rate at five years is 69%, but it varies according to the extent of the disease. Relevant to this, the survival rate at

one year is 90% for cutaneous disease but only 70% for visceral forms, which require treatment with chemotherapeutic agents. Interestingly, patients with KS who have heart or liver transplants appear to have a shorter survival than those with kidney allografts. Two epidemiologic studies, from the Cincinnati Transplant Tumor Registry and from the Collaborative Transplantation Research Group of the Ile de France, respectively, reported a higher incidence of KS in kidney and liver allograft recipients, with smaller incidence of recipients of other solid organs, and showed the rarity of KS occurrence in BM transplant patients. In particular, the incidence of KS in renal transplant patients is increased 400- to 500-fold over that seen in a control population of the same ethnic origin. HHV8 is not ubiquitous in the general population, with seroprevalence rates being very low in U.K. and U.S., and higher only in certain geographic areas of the world (the Mediterranean area, Eastern Europe, and sub-saharan Africa) with a known high incidence of the classic and endemic forms of KS. Similarly, the incidence of the post-transplant form of KS varies in different ethnic groups, being higher in those patients which originate from endemic areas for HHV-8 infection. Consistent with this, KS has been reported to develop in less than 1% of organ transplant recipients in two large patient series studied in Canada and France, being the KS patients of Mediterranean, African and Central European ethnic origin. Higher incidence rates of post-transplant KS have been detected in Israel (2.4%) and in Saudi Arabia (5.3%). Despite similar immunosuppressive regimens, KS was more frequent in transplant patients from Southern (2.98%) compared with Northern Italy (1.6%), reflecting the distribution of HHV8 seroprevalence rates in the same Italian regions. On the basis of only serologic test results, earlier studies suggested that post-transplant KS is primarily due to HHV8 reactivation in the recipient in areas where it is endemic and to primary infection with a virus transmitted from the donor in areas where it is not endemic. Subsequent studies from our and other groups, with findings based not only on serological data but also on molecular tracing of the viral infection, have shown that organ-related transmission of HHV8 is more common than has previously thought. Consistent with this, a careful analysis of the findings from 18 independent serological and/or molecular studies of HHV8 infection in 54 cases of post-transplant KS, has shown that in more than one third of them (20 of 54 cases) the virus was found to have come from the donor. The growing new information about HHV8 transmission and pathogenic potential in the setting of transplantation in turn spurs the crucial health issue of whether or not including the screening of potential organ donors for this new virus.

Knowledge of the HHV8 status of the graft donors and recipients could guide the proper use of HHV8 positive allografts and necessarily should prompt prophylactic treatment of transplant recipients. Relevant to this, our group has been coordinating a national effort in collaboration with the Nord Italian Transplant (NIT) and the Gruppo Italian Trapianto di Midollo (GITMO) to screen the solid organ and the BM donors and their paired recipients for HHV8, by means of serologic and molecular methods, in order to assess the frequency of HHV8 primary infection and reactivation, and associated clinical manifestations, including KS, in the setting of transplant patients. These data showed that the prevalence of HHV-8 infection in the group of renal and BM/PBSC donors is similar to that found in the general population, confirming that the prevalence of HHV-8 infection is a significant phenomenon in our country. The prevalence of HHV-8 infection in renal transplant patients showed an increase from 10% at the time of transplantation to 16% after transplantation. This increase suggests that a significant number of renal recipients has become infected with HHV-8 following the transplantation procedure. However, although we demonstrated the organ-related transmission of HHV-8 from the donor to two recipients, in our series most of the matched kidney donors resulted HHV-8 negative, suggesting that routes of transmission other than the transplanted organs should exist (saliva of HHV-8 positive family members etc.). The follow-up of these recipients in the next year will show whether HHV-8 primary infection and/or reactivation in these patients is related to clinical manifestations. In the setting of BM/PBSC transplantation, the prevalence of HHV-8 infection showed a decrease from 8% at the time of transplantation to 5%. This finding may be explained with the eradication of the HHV-8 carrier state following the myeloablation procedures (chemotherapy and total body irradiation) inherent with this kind of transplantation, while, conversely, the immunosuppressive regimen used in solid organ recipients do not destroy the cellular reservoir of the virus, which in turn may reactivate. Moreover, a method of Q-PCR has been validated, establishing a good correlation between the kinetics of the viral load and the clinical manifestations.

In the same study, we have used a combination of molecular and immunohistochemical methods to show that individual HHV8 infected neoplastic cells in the post-transplant KS lesions renal transplant patients, harboured either genetic or antigenic markers of their matched donors. In particular, to evaluate the donor origin of the cells forming the KS proliferation, we used PCR to analyse the sex (chromosome Y) and/or genetic markers (HLA DR4,7,8,11) of the donor on single microdissected cells, selected on the basis of their

morphological and immunophenotypical features (CD34+; HHV8/LANA-1+). In order to validate the identification of Y chromosome DNA by PCR on the tumor cells, we performed DNA in situ hybridization for the human Y and X chomosomes. We also assessed whether the microdissected KS tumors from patients were of donor or recipient origin using an identity testing PCR kit that amplifies nine microsatellite loci (short tandem repeats - STR). Furthermore, in order to evaluate the distribution of the donor and recipient HLA antigens in the skin biopsy from one patient with KS, we also performed a double immunofluorescence with antibodies directed against donor HLA A30-31 and recipient HLA23-24, respectively: whereas the epidermis and the subepidermal vessels only stained with anti-HLA A23-24, the tumor showed a prevalent expression of A30-31 in the endothelial cells. In conclusion, we detected the presence of sex and genetic markers of the matched donors in the neoplastic HHV-8 infected cells within the KS lesions from five of eight renal recipients. These lesions may be thus similar to those of post-transplant lymphoproliferative diseases occurring in recipients of BM grafts, which are known to result from the expansion of Epstein-Barr virus infected B cells of donor origin. In conclusion, our demonstration that the still elusive KS progenitor cells may be transmitted through solid grafts, provides a rationale for exploring the therapeutic use of donor HHV8 specific cytotoxic T cells.

The occurrence of either primary effusion lymphoma (PEL) or of multicentric Castleman's disease (MCD), or plasmablastic lymphomas, which are the only lymphoproliferative diseases known to be consistently associated with HHV-8 infection in HIV positive subjects, has so far been rarely reported in the setting of transplantation. Only two cases of PEL have been described, one pleural and one peritoneal, both of which have occurred in heart recipients originating from endemic areas (one from Haiti and one from Italy), 4 and 8 years respectively after transplantation. In one case EBV infection was also documented. In one patient KS and persistent polyclonal plasmacytic infiltrates involving multiple organs, including the cardiac allografts, preceded the occurrence of PEL. The clinical course was very poor, being survival of 3 and 6 months respectively, despite reduction of immune-suppression in both cases and the administration of chemotherapy in one. One case of MCD of PC type has been reported to occur 13 months after renal transplantation, in a Northern Italian woman, who subsequently developed also KS. Recently, the occurrence of EBV negative, HHV-8 positive, monoclonal, lymphoproliferative disease of polymorphic type has been reported to occur in a HHV-8 seronegative Jewish man, nine months after receiving a kidney from his HHV-8 seropositive father. KS and haemolytic anemia were also present, and chemotherapy led to clinical remission, which lasted 22 months. Additionally, two cases of HHV-8 positive and EBV negative polyclonal lymphoid proliferations have been described, in association with KS. The first patient developed, two months after orthotopic liver transplantation, recurrent pleural effusions and lymphadenopathy with hyperplastic features, similar to those occurring in MCD. The second patient presented, 2 years after renal transplantation, systemic lymphadenopathy and hepatosplenomegaly, secondary to diffuse infiltration by polyclonal plasma cells and plasmacytoid B cells.

As mentioned earlier, one of the major clinical issues, related to the management of patients with post-transplant KS, is represented by the need for a laboratory test which could allow and/or confirm a prompt and early diagnosis, and even more, which could bear a high predictive value for the development of KS or other HHV-8 related neoplastic diseases (i.e. lymphoproliferative diseases, such as PEL and MCD), in HHV-8 positive recipients and in HHV-8 negative recipients receiving a graft from a HHV-8 positive donor. The identification of such a laboratory test, would allow the development of strategies of pre-emptive therapies, i.e. therapies targeted to those patients at particularly high risk of developing HHV-8 related neoplastic diseases, based on laboratory results with high predictive value. Data from the literature suggest that quantification of HHV-8 load in the peripheral blood could represent a useful tool for monitoring transplant recipients with HIV infection, but no information are available on the predictive value of HHV-8 viral load determination in HHV-8 positive patients, without overt KS, in terms of their risk of developing KS or other HHV-8 related diseases. Moreover, in the setting of AIDS KS, very recent data based on the analysis of T cell responses to HHV-8 antigens by EliSpot, have shown a nice correlation between the increase of immune response, the decrease of HHV-8 viral load and the regression of KS. However, again, no information are yet available in the setting of post-transplant KS. Moreover, preliminary data from our laboratory on few HIV negative patients with iatrogenic KS, have shown a good correlation between the KS course and the immune response against three different HHV-8 antigens, but not with the determination of the viral load, by quantitative PCR, which has remained stable over time. This reinforces the need for further studies on a larger series of transplant patients, comparing the two methods in order to assess their sensitivity and sensibility.

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