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Prevalence of Human Herpesvirus-6 Chromosomal Integration (CIHHV-6) in Italian Solid Organ and Allogeneic Stem Cell Transplant Patients

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The unique phenomenon of human herpesvirus-6 (HHV-6) chromosomal integration (CIHHV-6) may account for clinical drawbacks in transplant setting, being misinterpreted as active infection and leading to unnecessary and potentially harmful treatments. We have investigated the prevalence of CIHHV-6 in 205 consecutive solid organ (SO) and allogeneic stem cell transplant (alloSCT) Italian patients. Fifty-two (38.5%) of 135 solid organ transplant (SOT) and 16 (22.8%) of 70 alloSCT patients resulted positive for plasma HHV-6 DNA by real-time polymerase chain reaction. Seven SOT and three alloSCT patients presented HHV-6-related diseases, requiring antivirals. Two further patients (0.9%) were identified, presenting high HHV-6 loads. The quantification of HHV-6 on hair follicles disclosed the integrated state, allowing the discontinuation of antivirals. Before starting specific treatments, CIHHV-6 should be excluded in transplant patients with HHV-6 viremia by the comparison of HHV-6 loads on different fluids and tissues. Pretransplantation screening of donors and recipients may further prevent the misdiagnosis of CIHHV-6.

Key words: Allogeneic stem cell transplantation, chromosomal integration, human herpesvirus 6, prevalence, solid organ transplantation

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Human herpesvirus-6 (HHV-6) is an ubiquitous β-herpesvirus existing in two variants, namely variant A and B (1). It is the causative agent of the sixth disease of the childhood and infects, virtually, all children within the first few years of life (1). Like other herpesviruses, after the primary infection, HHV-6 establishes latency, from which it may reactivate and cause illnesses in immunosuppressive states (2,3). Approximately 60% of solid organ transplant (SOT) and 40% of allogeneic stem cell transplant (alloSCT) patients experience HHV-6 reactivation, mainly of variant B, with variant A accounting for 2–3% of the events (2,3). Primary infection may also occur in the small fraction of seronegative patients undergoing transplantation, usually as a result of the transmission of the virus from the donor through the infected organ (2–4). Fever, bone marrow suppression, encephalitis, pneumonitis and hepatitis have been reported as direct sequelae of HHV-6 active infection, while graft versus host disease, delayed platelet engraftment and susceptibility to opportunistic agents have been appreciated to a greater extent as indirect sequelae (2–5).

A lesser-recognized form of HHV-6 latency is the integration of the viral genome in a host chromosome (CIHHV-6) (6). This phenomenon features a high viral copy number either in whole blood (>6 $\log_{10}$ HHV-6 copies/mL) or sera (>3.5 $\log_{10}$ copies/mL) or other body sites (>4 $\log_{10}$ copies/hair follicle or/mL of cerebrospinal fluid), as a consequence of inheritance of viral sequences through the germ line and their retainance in every nucleated cell (7). The prevalence of CIHHV-6 has been resulted 0.21% in healthy subjects from Japan and ranging from 0.8% to 3% in the general population from United Kingdom, while it is 1.5% in children treated for acute leukemias in the Czech Republic (8–10). Although the integrated viral genome may be transcriptionally active, to date, no replication has been reported either *in vitro* or *in vivo* (11). CIHHV-6 is commonly considered neither affecting the host health status nor requiring clinical intervention (11), but its clinical significance remains largely unknown. Only one case of CIHHV-6 and concurring encephalomyelitis, subsequently relieved by antivirals, has so far been reported in an immunocompetent

subject (12). Essentially, CIHHV-6 may confound the laboratory diagnosis of HHV-6 active infection prompting possibly unnecessary and harmful antiviral treatment, as recently published (13,14).

Here, we report the prevalence of CIHHV-6 in a cohort of SOT and alloSCT patients from a single Italian center and discuss issues related to the clinical manifestations and the management of the patients carrying CIHHV-6.

Patients and Methods

Three hundred and forty-three SOT and 78 alloSCT consecutive patients from the Liver and Multivisceral Transplant Center and the Section of Hematology of the University Hospital of Modena were evaluated for the study from April 2000 to April 2008. The clinical characteristics of the patients are reported in Table 1.

Plasma samples, ficoll-separated peripheral blood mononuclear cells, whole peripheral and bone marrow blood, bronchoalveolar lavage fluid, cerebrospinal fluid and tissue specimens, namely bone marrow trephine biopsy, gastric and cutaneous biopsies and hair follicles were collected and screened for HHV-6 active infection in the presence of clinical manifestations for which a viral etiology was suspected, either in the early posttransplant period or during the outpatient controls as a part of a routine virologic screening. On the same occasions, cytomegalovirus (CMV) and Epstein–Barr Virus (EBV) were investigated by quantitative real-time polymerase chain reaction (qrt-PCR) on plasma and whole blood, and CMV also by pp65 antigenemia assay on pheripheral blood polymorphonuclear cells, as described (4). SOT recipients at high risk for CMV infections, because CMV seronegative and receiving a CMV seropositive graft, underwent antiviral prophylaxis with oral valganciclovir 450 mg bis in die (b.i.d.) until the third month posttransplantation. Until 1 year posttransplantation, all the other SOT patients received oral acyclovir, at a dose of 400 mg b.i.d., while SCT patients received acyclovir at a dose of 500 mg/m^2 ter in die (t.i.d.) endovenously during the first 30 day posttransplantation, then orally. The median follow-up was 35.7 $\pm$ 29 months. The study was approved by the local Ethical Committee. Each patient gave the informed consent for the study.

CMV, EBV and HHV-6 viral loads have been quantified by commercially available kits (Nanogen Advanced Diagnostics, Turin, Italy), able to detect a minimal quantity of 10 genome Equivalent of each virus per reaction, as previously reported (4). Cases suspected to carry CIHHV-6 were confirmed by an independent laboratory with a different quantitative PCR method as previously reported (15). HHV-6 variant characterization was performed by restriction analysis of the HHV-6 PCR product and also by a highly sensitive primer-specific PCR assay, as described (4). The HHV-6 variant specific PCR has demonstrated a sensitivity threshold of about 10 copies/well for HHV-6 variant A and 1 copy/well for HHV-6 variant B and the assay may detect and identify one variant in the presence of more than 1000 times higher concentrations of the other variant in virus mixtures (16). HHV-6, CMV and EBV associated diseases have been defined as reported (Ref. 1–3, Supporting Information).

Biopsy specimens from patients were studied with hematoxylin and eosin staining for routine histologic studies. Immunohistochemical analyses with standard immunoperoxidase-staining procedures were performed with the use of the mouse monoclonal antibodies, as previously described (4).

Results

During the entire follow-up period, 292 episodes of HHV-6 viremia (218 in SOT and 74 in SCT setting, respectively),

Table 1: Clinical characteristics of the patients

SCT Patients

Pts number	Median age (years)	Disease (%)	Donor sibling/unrelated	Stem cell source	ATG in induction immunosupression (%)	GVHD acute/ chronic	GVHD grading II-III/IV	HHV-6 screening (%)
78	45	ALL 14 (18) AML 22 (28) MDS 5 (6) MPD 12 (15) Lymphoma 13 (17) MM 11 (14) Solid tumor 1 (2)	63/15	BM 9 PB 67 BM + PB 1 CB 1	21 (27)	19/18	17/1	70 (90)

SOT patients

Pts number	Median age (years)	Type of transplant (%)	Induction immunosuppressive regimen (%)	Maintenance immunosuppressive regimen (%)	Rejection acute/ chronic	Rejection treatment	HHV-6 screening (%)
343	57	Liver 303 (88) Liver–kidney 15 (4.5) Liver–heart 1 (0.2) Liver–small bowel 3 (0.8) small bowel 21 (6.5)	ATG 3 (0.8) CyA 77 (23) PDN 1 (0.2) MMF 4 (1) FK 258 (75)	CyA 77 (23) FK 255 (74) Sirolimus 11 (3)	152/15	PDN 128 OKT3 21 CyA 7 FK 11	135 (39)

Pts = patients; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; MDS = myelodysplastic syndrome; MPD = myeloproliferative disorder; MM = multiple myeloma; BM = bone marrow; PB = peripheral blood; CB = cord blood; ATG = antithymocyte globulin; GVHD = graft versus host disease; HHV-6 = human herpes virus six; CyA = cyclosporine A; PDN = prednisone; MMF = mycophenolate mofetil; FK = tacrolimus; OKT3 = antiCD3 antibody.

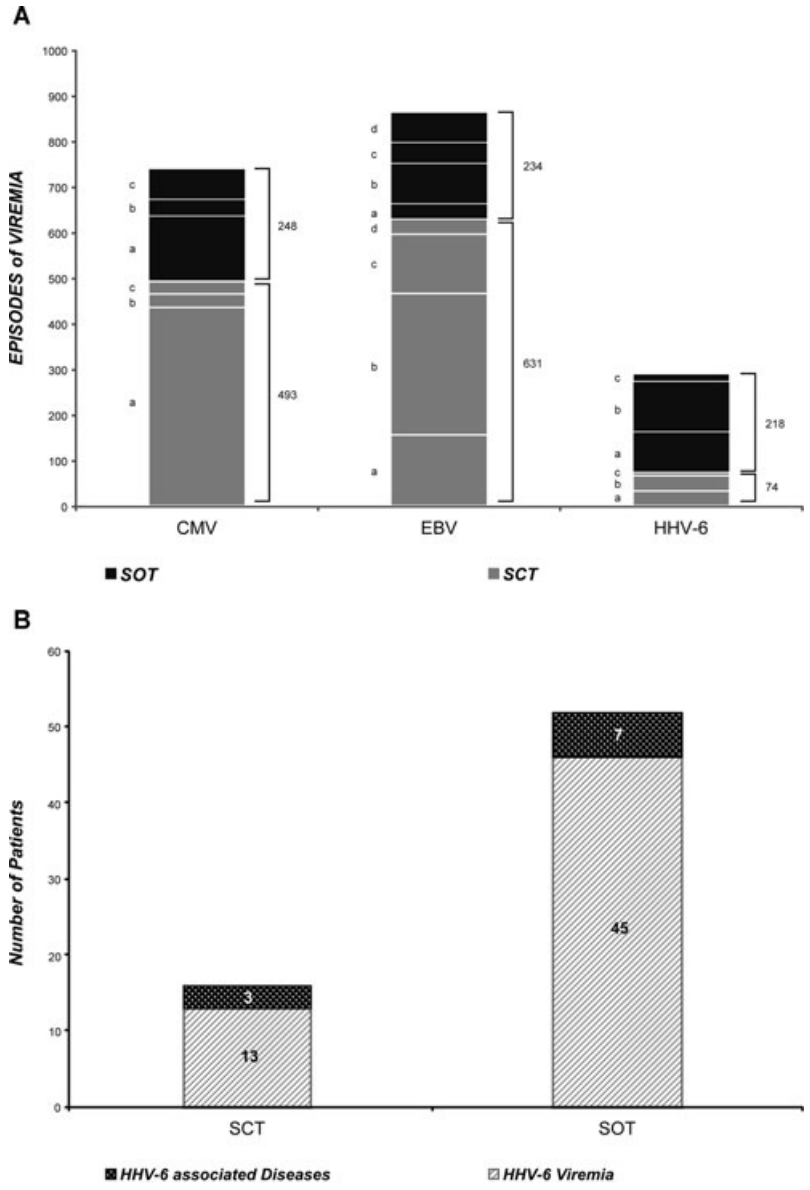

Figure 1: Frequency of episodes of herpesvirus viremia and of HHV-6 infection cases during the study period. (A) CMV, EBV and HHV-6 viremias detected during the entire follow-up both in SCT (gray columns) and SOT (black columns) patients. The episodes of viremia have been grouped based on the amount of viral loads to highlight the percentage of very mild (only EBV), mild, moderate and severe viral active infections. CMV column, segment a: percentage of episodes of antigenemia ranging from 1 to 5 CMV nuclei/200 000 polymorphonuclear leukocytes in SCT (gray; 88.4%) and SOT (black; 57.8%) patients; segment b: percentage of episodes of antigenemia ranging from 6 to 10 CMV nuclei/200 000 polymorphonuclear leukocytes in SCT (gray; 6%) and SOT (black; 14.8%) patients; segment c: percentage of episodes of antigenemia with >10 CMV nuclei/200 000 polymorphonuclear leukocytes in SCT (gray; 5.6%) and SOT (black; 27.4%) patients. EBV column, segment a: percentage of episodes of viremia ranging from >20 to 100 EBV genome copies/mL of whole blood in SCT (gray; 25.2%) and SOT (black; 14.2%) patients; segment b: percentage of episodes of viremia ranging from 101 to 1000 EBV genome copies/mL of whole blood in SCT (gray; 48.9%) and SOT (black; 38%) patients; segment c: percentage of episodes of viremia ranging from 1001 to 4000 EBV genome copies/mL of whole blood in SCT (gray; 20.6%) and SOT (black; 19.6%) patients; segment d: percentage of episodes of viremia with >4000 EBV genome copies/mL of whole blood in SCT (gray; 5.3%) and SOT (black; 28.2%) patients. HHV-6 column, segment a: percentage of episodes of viremia ranging from ≥20 to 100 HHV-6 genome copies/mL of plasma in SCT (gray; 43.6%) and SOT (black; 40.7%) patients; segment b: percentage of episodes of viremia ranging from 101 to 1000 HHV-6 genome copies/mL of plasma in SCT (gray; 45.8%) and SOT (black; 50.8%) patients; segment c: percentage of episodes of viremia ranging with >1000 HHV-6 genome copies/mL of plasma in SCT (gray; 10.6%) and SOT (black; 8.5%) patients. (B) Number of patients with HHV-6 viremia (dotted gray columns) and HHV-6 associated diseases (dotted black columns) within the whole transplant population. SCT = stem cell transplant; SOT = solid organ transplant.

were recorded (Figure 1A). Total numbers of patients screened for HHV-6 DNA either on body fluids and tissue specimens, were 135 out of 343 (39%) SOT and 70 out of 78 (90%) alloSCT (1014 samples analyzed; median of 4.9 sample/patient). Fifty-two (39%) out 135 SOT and 16 (23%) out of 70 alloSCT patients presented at least one positive sample for HHV-6.

Seven (13.4%) out of 52 SOT and 3 (18%) out of 16 alloSCT patients presented symptoms and signs consistent with HHV-6-related diseases, namely thrombocytopenia 2, fever 2, fever and lymphadenopaties 1, neutropenia and thrombocytopenia 4, syncytial giant cell hepatitis 1 (Figure 1B). This latter patient has been previously reported (4). The patients with HHV-6-related diseases presented HHV-6 loads included between 20 and 1000 HHV-6 genome copies (gc)/mL of plasma (Figure 1A). Nineteen (36.5%) out of 52 SOT and 4 (25%) out of 16 SCT patients with HHV-6 viremia presented CMV coinfection. Patients with concomitant HHV-6 and CMV viremia presented neither higher values of CMV antigenemia nor worse clinical course than patients with CMV alone. The percentage of HHV-6 coinfection, either in SOT or SCT patients with CMV viremia and their clinical outcome are consistent with those recently reported by Humar et al. in SOT patients alone and are in agreement with the raised possibility that coinfections may only reflect a more intense immunosuppressive states, without a clear clinical significance (17).

Two (0.9%) out of 205 patients, one alloSCT and one SOT patient, respectively, demonstrated HHV-6 loads consistent with CIHHV-6. Their clinical outcome has been reported in detail, below.

Seven hundred and forty-one episodes of CMV viremia (493 in SOT and 248 in SCT setting, respectively) were registered (Figure 1A). All but five patients were treated with antivirals on a preemptive basis, the remaining five cases presented CMV diseases. Eight hundred and sixty-five episodes of Epstein–Barr virus viremia (631 in SOT and 234 in SCT setting, respectively) were also detected (Figure 1A). Eleven out of 70 alloSCT patients with EBV viremia were treated with preemptive rituximab, according to studies suggesting that early treatment may avoid progression to posttransplant lymphproliferative disorders (PTLDs) and thus improve the outcome of EBV associated diseases (18). All the 11 patients had complete resolution of their elevated viremia without further complications. Four out of 135 SOT patients developed PTLDs, of which two resulted EBV positive and two EBV negative on immunohistochemical and molecular studies. All four cases were treated with rituximab alone and only two patients responded.

Case 1

A 60-year-old Caucasian man underwent a peripheral blood stem cell transplant from a human leukocyte antigen-matched, unrelated donor because of high-risk myelodysplastic syndrome. Immunsuppressive regimen consisted of antithymocyte globulin (10 mg/kg days from −4 to −2), methotrexate (10 mg/m^2 day +3 and +6), cyclosporine from day −1 (blood trough level 200–400 ng/mL). On day +21 the patient presented maculopapular, somewhere confluent, erythematous exanthema involving the trunk and the limbs and his peripheral white blood cell count demonstrated a delayed recovery, being leukocytes 340/mm^3. HHV-6 DNA was disclosed on plasma [6478 gc/mL] (Figure 2A). Bone marrow HHV-6 DNAemia also resulted extremely elevated (374 682 gc/mL) (Figure 2B). Foscarnet was started at 60 mg/kg t.i.d. and a skin biopsy was performed (Figure 2A). The histologic examination revealed cutaneous grade II acute graft versus host disease (GVHD), and corticosteroids (2 mg/kg) were added. From day +43, acute GVHD improved, but HHV-6 was still detectable at high loads (Figure 2A). Steroids were tapered at 0.20 mg/kg and foscarnet continued. On day +58, nonetheless the HHV-6 loads persisted unmodified, either on plasma or bone marrow blood, foscarnet was reduced at the maintenance dosage (90 mg/day, 5 day/week) (Figure 2A). On day +97, HHV-6 persisted detectable at high level either on plasma or bone marrow blood, but the patient resulted asymptomatic and foscarnet was finally discontinued. On day +110, he complained severe gastric pain, dyspepsia and the skin rash reappeared. HHV-6 loads increased on plasma, while remained unmutated on bone marrow blood (Figure 1A). Gastric and skin biopsies demonstrated GVHD grade II to III. HHV-6 load was 138046 gc/10^5 cells, on gastric tissue, but immunohistochemical analysis failed to demonstrate HHV-6 productive infection of the gastric cells. Before restarting antiviral therapy, the quantification of HHV-6 on the patient's hair follicles was performed and resulted 5318242 gc/10^6 cells. The viral variant was typed as variant A. The patient was considered to carry CIHHV-6 and the antiviral treatment judged unnecessary. Currently, at day +259, bone marrow HHV-6 DNAemia persists at high level, while HHV-6, on plasma, has been reduced to 96 gc/mL, as a consequence of the improvement in peripheral blood chimerism from <10% donor origin on day +21 to 85% donor origin.

Case 2

In July 2007, a 43-year-old Caucasian man was hospitalized because of fever and bloody diarrhea. Seven years before admission, in December 2000, the patient had been referred to the hospital because of mental status changes, intermittent somnolence and confusion. He presented malnutrition and dysprotidemia because of chronic intestinal pseudo-obstruction. On that occasion, a lumbar puncture, revealed positivity for HHV-6 at 7992 gc/mL. Foscarnet at therapeutic dosage (60 mg/kg t.i.d.) and parenteral nutrition supplementation were started with improvement of symptoms and clinical conditions. On the same month,

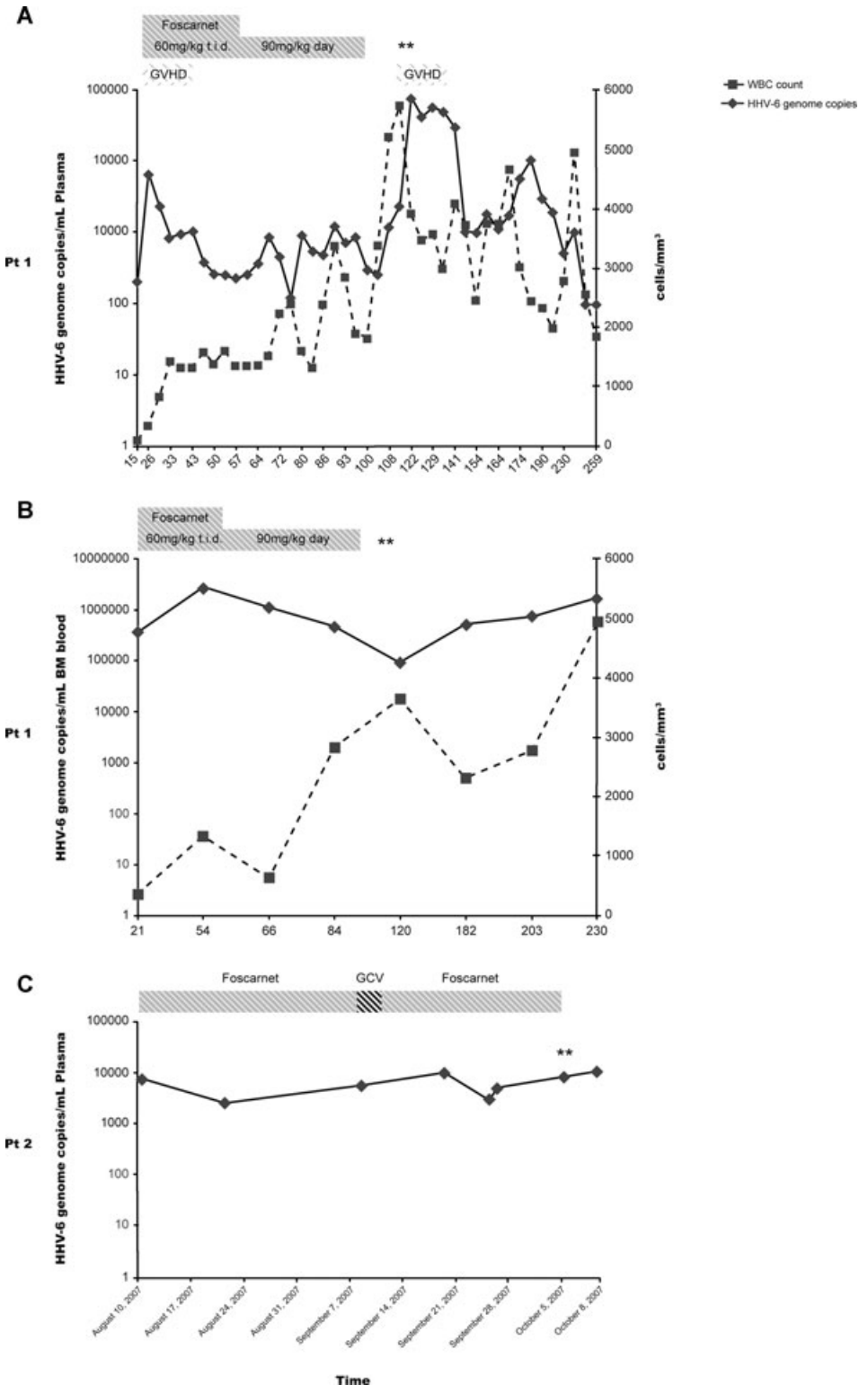

Figure 2: Time course of HHV-6 loads on different biologic fluids and their correlation with antiviral treatment in the two transplant patients with CIHHV-6. (A) Correlation between plasma HHV-6 DNA and WBC in the stem cell transplant patient. (B) Correlation between bone marrow blood HHV-6 DNA and WBC in the stem cell transplant patient. (C) Monitoring of the plasma HHV-6 DNA in the solid organ transplant patient. WBC = white blood cells; GVHD = graft versus host disease; GCV = ganciclovir; ** = detection of HHV-6 on hair follicles; t.i.d. = three times a day; CIHH-6 = HHV-6 chromosomal integration.

the patient received a small bowel transplantation, performed under antiviral treatment, because HHV-6 persisted detectable on body fluids. Immunosuppressive regimen consisted of alemtuzumab 0.3 mg/kg before transplant, after reperfusion, at day 3 and at day 7 and of maintenance with tacrolimus (trough blood level of 10–15 ng/mL during the first 3 months followed by 8–12 ng/mL, thereafter). The immediate postoperative course was complicated

by CMV reactivation, while HHV-6 remained stably detectable either on plasma or on whole blood. Foscarnet was replaced by gancyclovir (5 mg/kg b.i.d.). HHV-6 load persisted unmutated, while CMV antigenemia negativized. A diagnosis of chronic HHV-6 infection was made and the patient was discharged in good clinical conditions with oral gancyclovir 1 g t.i.d. In the following hospital admissions and in the periodic outpatient controls, HHV-6 remained always detectable on body fluids. Antiviral treatment was stably maintained and withdrawn only when the patient presented related severe side effects.

On the last admission, in July 2007, the patient's CMV antigenemia resulted positive and CMV was detected either by PCR or by *in situ* hybridization on intestinal biopsy. HHV-6 demonstrated positive on plasma with 7364 gc/mL (Figure 2C). Oral gancyclovir was replaced by foscarnet 60 mg/kg t.i.d. After 1 week, CMV antigenemia disappeared and the stool normalized. HHV-6 persisted on plasma at 2482 gc/mL. On the fourth week of treatment, although CMV detection remained persistently negative, HHV-6 resulted 5489 gc/mL on plasma. Foscarnet was replaced by endovenous gancyclovir at the dose of 5 mg/kg b.i.d (Figure 2C). Three days later, severe neutropenia developed, and foscarnet was reinstituted. HHV-6 persisted elevated on plasma, and the patient developed bilateral pneumonia. HHV-6, Pseudomonas aeruginosas and Candida glabrata were isolated from the bronchoalveolar lavage fluid and colystine and caspofungin were associated with antiviral treatment. HHV-6 loads on plasma further increased (Figure 1C). Patient's condition deteriorated, neutropenia persisted and a hematologic consultancy was requested. Granulocyte colony stimulating factor was started and the proposal of testing HHV-6 on hair follicles was suggested. The quantification of HHV-6 on hair resulted 11159420 gc/10^6 cells, consistent with CIHHV-6. The virus was identified as HHV-6 variant B. Foscarnet was withdrawn. Unfortunately, the patient died due to pseudomonas pneumonia few days later.

Discussion

This is the first survey on the prevalence of CIHHV-6 in transplant patients, including both SOT and SCT. The prevalence resulted 0.9%, consistent with that being found in the general population from other different geographical areas (8,9) and could possibly indicate the frequency of this phenomenon in the Italian population. However, due to the fact that the search for HHV-6 was left to the compliance of the referring physicians, only less than a half of the SOT recipients have been screened for HHV-6. This possibly makes estimating the true prevalence and the clinical value of CIHHV-6 unreliable and may have led to underdiagnosis of the CIHHV-6 phenomenon in the transplant series studied.

Only four cases of posttransplantation, CIHHV-6 have so far been described in the literature in hematologic patients undergoing alloSCT, as single case reports (13,19,20). All but one cases resulted from the transmission of the integrated HHV-6 from a donor to a recipient, without viral integration (13,19). The remaining case showed the opposite donor–recipient combination and the clinical outcome was characterized by the disappearance of HHV-6 DNA from the peripheral blood of the recipient soon after the engraftment of the donor blood cells at day +70, as a consequence of the complete replacement of the recipient's hemopoietic system with that of the integration-negative donor (20).

CIHHV-6 originated from the recipient in both our patients. The histories of our patients clearly demonstrate the clinical drawbacks of the CIHHV-6. Although no controlled trials of antiviral therapy in the course of HHV-6 active infections have so far been conducted, either *in vitro* data or a growing number of case reports and small series of patients have shown some potentiality of either ganciclovir or foscarnet in inhibiting HHV-6 replication (2,21). Actually, both antivirals may be initiated, especially when high levels of HHV-6 viremia are associated with clinical manifestations in immunosuppressed patients with a grade of recommendation classified as BIII (22). In fact, in the alloSCT patient, the high plasma and bone marrow blood viral loads associated with skin rash and cytopenias induced the incorrect diagnosis of HHV-6-related disease and the precipitous use of antiviral therapy. In the SOT patient, the positive result of PCR on cerebrospinal fluid associated with neurologic symptoms first, and later the persistence of high viral loads on plasma associated with respiratory symptoms, erroneously led the clinicians to administer prolonged and toxic antiviral treatment.

Furthermore, case 1 extends the spectrum of clinical manifestations of CIHHV-6 in SCT setting, by showing that: (1) episodes of GVHD may increase the value of HHV-6 load in plasma, (2) the persistence of high HHV-6 loads on bone marrow blood, also when the HHV-6 DNA in plasma is finally reducing. The former is, possibly, a consequence of the increased virus shedding from the immune destruction of recipient's cells by host's lymphocytes. This is due to the fact that in subjects carrying CIHHV-6, viral sequences are found in each cell that carries human chromosomal DNA and any inflammatory process, causing an increased cell turnover, may lead to variation of HHV-6 DNA in body fluids. The latter may, possibly, reflect different states of chimerism in different compartments, as in patients with CIHHV-6 of recipient origin, HHV-6 loads in plasma and whole blood are expected to drop, until almost complete disappearance, when nonintegrated donor's WBCs replace completely the integrated recipient's WBCs (20), while, in bone marrow blood, HHV-6 loads are maintained by the persistence of integrated recipient's adherent cells and/or remnant hematopoietic cells. Moreover, the very high HHV-6 loads detected in the gastric biopsy could

be interpreted as a further proof of the integrated state of our patient, given that HHV-6 is present in every nucleated cell in subjects with CIHHV-6. The possibility of upper gastrointestinal and ileocolonic HHV-6 infection, as reported in a series of SCT recipients and more recently in liver transplant patients (23,24), is very unlikely, even considering the absence of positivity for the antibody anti gp41/38, a HHV-6A early protein, in gastric cells on immunochemistry, reflecting the absence of the production of viral proteins and the resolution obtained with immunosuppressive treatment, without the use of antivirals. Of note, although the integrated genome of HHV-6 may transcribe viral genes, either spontaneously or under chemical stimulation, it has been reported that, upon the same stimuli, no proteins could be detected in cell cultures harboring CIHHV-6 (11,25).

In addition, case 2 represents the first-reported patient with CIHHV-6 in the SOT setting. Three (5%) out of 60 liver and two (3.8%) out of 52 renal transplant patients with persistent and strikingly high HHV-6 viral loads were described in two previous reports on the HHV-6 active infections in subjects undergoing SOT (ref in 13, 26) . These patients were supposed to represent cases of CIHHV-6 in a following paper by Clark et al. (13), but the appropriate screening to confirm the viral integration was never performed.

It has been reported that HHV-6 variant A is most commonly found in integrated cases (10,13,14,19,20). We found one patient carrying the variant A and the other the variant B. Although all the CIHHV-6 cases reported in SCT patients show variant A, the majority of integrated cases carries the viral variant B in the general population (8,9).

In conclusion, CIHHV-6 may occur with a significant frequency both in SCT and SOT patients. The results of molecular studies should be critically evaluated and the distinction between HHV-6 active infection and CIHHV-6 should be pursued by the comparison of viral loads on different biological fluids, because the finding of very high and persisting levels of HHV-6 DNA (>3.5log10 copies/mL in plasma or CSF, and >6log10 copies/mL in whole peripheral or bone marrow blood) at about one copy per cell are highly suggestive of CIHHV-6 rather than active infection (6–9). The determination of viral variant is useless for this scope, as either variant A or variant B have been found integrated. Viral isolation and the antigenemia test are still time consuming and not always reliable. The search for HHV-6 DNA in hair follicles, nails or tissue specimens should be performed to confirm the occurrence of CIHHV-6. These latter tests and a proper clinical evaluation may preclude unnecessary and potentially harmful treatments. Nonetheless, also considering the report by Troy et al. (12), the antiviral treatment may not be completely dismissed, also in the case of CIHHV-6, if the clinical symptoms are suggestive of HHV-6-related diseases and all other possible causative agents have been excluded. Larger studies urge to be performed, to understand the clinical implications of the phenomenon of CIHHV-6. Finally, a wider pretransplantation screening, including also HHV-6 quantification, of organ donors and recipients, could prevent misinterpretation of CIHH-6 in the recipients after transplantation and, if applied in a prospective study from a specific geographical area, may help to definitively estimate the prevalence of CIHHV-6 phenomenon in transplant patients in a given country.

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References

1. Zerr DM, Meier AS, Selke SS, Frenkel LM, Huang ML, Wald A. A population-based study of primary human herpesvirus 6 infection. N Engl J Med 2005; 352: 768–776.
2. Clark DA, Griffiths PD. Human herpesvirus-6: Relevance of infection in the immunocompromised host. Br J Haematol 2003; 120: 384–395.
3. Zerr DM, Corey L, Kim HW, Huang M-L, Nguy L, Boeckh M. Clinical outcome of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. Clin Infect Dis 2005; 40: 932–940.
4. Potenza L, Luppi M, Barozzi P et al. HHV-6A in syncytial giant-cell hepatitis. N Engl J Med 2008; 359: 31–40.
5. Rogers J, Rohal S, Carrigan D et al. Human herpesvirus-6 infection in liver transplant recipients: Role in pathogenesis of fungal infections, neurologic complications and outcome. Transplantation 2000; 69: 2566–2573.
6. Luppi M, Marasca R, Barozzi P et al. Three cases of human herpesvirus-6 latent infection: Integration of viral genome in peripheral blood mononuclear cell DNA. J Med Virol 1993; 40: 44–52.
7. Ward KN, Leong HN, Nacheva EP et al. Human herpesvirus 6 chromosomal integration in immunocompetent patients results in high levels of viral DNA in blood, sera, and hair follicles. J Clin Microbiol 2006; 44: 1571–1574.
8. Leong HN, Tuke PW, Tedder RS et al. The prevalence of chromosomally integrated human herpesvirus 6 genomes in the blood of UK blood donors. J Med Virol 2007; 79: 45–51.
9. Tanaka-Taya K, Sashihara J, Kurahashi H et al. Human herpesvirus 6 (HHV-6) is transmitted from parent to child in an integrated form and characterization of cases with chromosomally integrated HHV-6 DNA. J Med Virol 2004; 73: 465–473.
10. Hubacek P, Muzikova K, Hrdlickova A et al. Prevalence of HHV-6 integrated chromosomally among children treated for acute lymphoblastic or myeloid leukemia in the Czech Republic. J Med Virol 2009; 81: 258–263.
11. Clark DA, Tsao EHF, Leong HN, Ward KN, Nacheva EP, Griffiths PD. Reply to Boutolleau et al and Luppi et al. J Infect Dis 2006; 194: 1021–1023.

12. Troy SB, Blackburn BG, Yeom K, Caulfield AK, Bhangoo MS, Montoya JG. Severe encephalomyelitis in an immunocompetent adult with chromosomally integrated human herpes virus 6 and clinical response to treatment with foscarnet plus ganciclovir. Clin Infect Dis 2008; 47: e93–e96.
13. Clark DA, Nacheva EP, Leong HN et al. Transmission of integrated human herpesvirus 6 through stem cell transplantation: Implications for laboratory diagnosis. J Infect Dis 2006; 193: 912–916.
14. Hubacek P, Maalouf J, Zajickova M et al. Failure of multiple antivirals to affect high HHV-6 DNAaemia resulting from viral chromosomal integration in case of severe aplastic anemia. Haematologica 2007; 92: e98–e100.
15. Gautheret-Dejean A, Manichanh C, Thien-Ah-Koon F et al. Development of a real-time polymerase chain reaction assay for the diagnosis of human herpesvirus-6 infection and application to bone marrow transplant patients. J Virol Methods 2002; 100: 27–35.
16. Boutolleau D, Duros C, Bonnafous C et al. Identification of human herpesvirus 6 variants A and B by primer-specific real-time PCR may help to revisit their respective role in pathology. J Clin Virol 2006; 35: 257–263.
17. Humar A, Asberg A, Kumar D et al. An assessment of herpesvirus co-infections in patients with CMV disease: Correlation with clinical and virologic outcomes. Am J Transplant 2009; 9: 374–381.
18. Weinstock DM, Ambrossi GG, Brennan C, Kiehn TE, Jakubowski A. Preemptive diagnosis and treatment of Epstein–Barr virus associated post transplant lymphoproliferative disorder after hematopoietic stem cell transplant: An approach in development. Bone Marrow Transplant 2006; 37: 539–546.
19. Kamble RT, Clark DA, Leonag HN, Heslop HE, Brenner MK, Garrum G. Transmission of integrated human herpesvirus-6 in allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2007; 40: 563–566.
20. Hubacek P, Hyncicova K, Muzikova K, Cinek O, Zajac M, Sedlacek P. Disappearance of pre-existing high HHV-6 DNA load in blood after allogeneic SCT. Bone Marrow Transplant 2007; 40: 805–806.
21. Zerr D, Gupta D, Huang M-L, Carter R, Corey L. Effect of antivirals on human herpesvirus 6 replication in hematopoietic stem cell transplant recipients. Clin Infect Dis 2002; 34: 309–317.
22. [No Authors listed] Other herpesviruses: HHV-6, HHV-7, HHV-8, HSV-1 and -2, VZV. Am J Transplant 2004; 4(Suppl 10):66–71.
23. Hentrich M, Oruzio D, Jäger G et al. Impact of human herpesvirus-6 after haematopoietic stem cell transplantation. Br J Haematol 2005; 128: 66–72.
24. Halme L, Arola J, Höckerstedt K, Lautenschlager I. Human herpesvirus 6 infection of the gastroduodenal mucosa. Clin Infect Dis 2008; 46: 434–439.
25. Daibata M, Taguchi T, Taguchi H, Miyoshi I. Integration of human herpesvirus 6 in a Burkitt's lymphoma cell line. Br J Haematol 1999; 102: 1307–1313.
26. Griffiths PD, Ait-Khaled M, Bearcroft CP et al. Human herpesviruses 6 and 7 as potential pathogens after liver transplant: Prospective comparison with the effect of cytomegalovirus. J Med Virol 1999; 59: 496–501.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Reference 1: Humar A, Michaels M; AST ID Working Group on Infectious Disease Monitoring. American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. Am J Transplant 2006; 6: 262–274.

Reference 2: Ljungman P, de la Camara R, Cordonnier C, Einsele H, Engelhard D, Reusser P et al. Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with hematological malignancies and after SCT. Bone Marrow Transplant 2008; 42: 227–240.

Reference 3: Styczynski J, Reusser P, Einsele H, de la Camara R, Cordonnier C, Ward KN et al. Management of HSV, VZV and EBV infections in patients with hematological malignancies and after SCT: guidelines from the Second European Conference on Infections in Leukemia. Bone Marrow Transplant 2008 Dec 1. PMID: 19043458 [Epub ahead of print].

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