

# Analysis of human herpes virus 6 infections with a quantitative, standardized, commercial kit in pediatric stem cell transplant recipients after transplantation

Derya Mutlu,<sup>a</sup> Vedat Uygun,<sup>b</sup> Hatice Yazisiz,<sup>c</sup> Gulsun Tezcan,<sup>b</sup> Volkan Hazar,<sup>b</sup> Dilek Colak<sup>d</sup>

From the <sup>a</sup>Akdeniz University, Faculty of Medicine, Medical Microbiology Department, Antalya, Turkey; <sup>b</sup>Akdeniz University, Faculty of Medicine, Pediatric Hematology-Oncology Department, Antalya, Turkey; <sup>c</sup>Ataturk State Hospital, Microbiology and Clinical Microbiology Laboratory, Antalya, Turkey; <sup>d</sup>Akdeniz University School of Medicine Medical Microbiology Department, Division of Virology, Antalya, Turkey

Correspondence: Dilek Colak · Akdeniz University, Faculty of Medicine, Medical Microbiology Department Dumlupinar Blv. Antalya Turkey 07070 · T: +902422496911 F: +902422262823 · dcolak@akdeniz.edu.tr

Ann Saudi Med 2014; 34(1): 6-11

DOI: 10.5144/0256-4947.2014.6

**BACKGROUND AND OBJECTIVES:** The aim of the study was to assess the incidence and clinical relevance of active Human Herpes Virus 6 (HHV6) infections in pediatric patients after allogeneic stem cell transplantation.

**DESIGN AND SETTINGS:** Retrospective analysis of samples prospectively collected at Akdeniz University Medical Faculty Hospital, Antalya, Turkey, between May 2006 and July 2007 from 15 pediatric patients with allogeneic hematopoietic stem cell transplantation (HSCT).

**SUBJECTS AND METHODS:** A commercial quantitative real-time polymerase chain reaction kit was used to analyze plasma samples collected from 15 pediatric allogeneic HSCT recipients.

**RESULTS:** HHV6 DNA was found positive in 8 (53%) patients. HHV6 DNA levels above 1000 copies/mL were found only in 2 patients and they were also consecutively positive for HHV6 DNA. Age at transplantation, use of ATG, and receiving grafts other than HLA identical siblings increased the risk, with a statistically significant difference, of having HHV6 reactivation with levels exceeding 1000 copies/mL (*P* values, respectively, *P*=.03, .001, .025). Active HHV6 infections with HHV6 viremia levels higher than 1000 copies/mL were associated with subsequent delayed platelet engraftment (*P*=.001), acute graft versus host disease (*P*=.001), skin rash, and fever of unknown origin.

**CONCLUSION:** More than half of pediatric allogeneic HSCT patients develop active HHV6 infection, and especially in patients with high viremic loads, the infection can result in serious clinical situations. A clinically significant cutoff value for viremia seems to be necessary to predict serious clinical complications.

Primary infection of human herpes virus 6 (HHV6) occurs during early childhood, and seropositivity rates or HHV6 DNA detection in saliva or peripheral blood mononuclear cell (PBMC) samples reach approximately 95% among adults.<sup>1-3</sup> Horizontal transmission within families through saliva is accused of being the primary route of transmission.<sup>4</sup> After primary infection, HHV6 remains latent in multiple organs and cells including salivary glands, lymph nodes, and PBMCs.<sup>5-7</sup>

Hematopoietic stem cell transplantation (HSCT) is widely used for a variety of hematologic, immunologic, and neoplastic disorders.<sup>8</sup> Administration of immu-

nosuppressive drugs to prevent graft versus host disease (GVHD) and rejection of the donor graft after HSCT make the patient more susceptible and vulnerable for viral infections. In stem cell transplant recipients, HHV6 infection rates were found to be as high as 41.9%.<sup>9</sup>

HHV6 infections are associated with a variety of clinical conditions including GVHD, delayed platelet and monocyte engraftment, central nervous system (CNS) disorders, and CMV and EBV reactivations.<sup>8,10,11</sup>

In this study the incidence and clinical relevance of active HHV6 infections in pediatric allogeneic stem cell transplant recipients were assessed with a commercial

quantitative real-time polymerase chain reaction (PCR) assay for 3 months after transplantation.

## SUBJECTS AND METHODS

### Patients

Fifteen children who had allogeneic stem cell transplantation in Akdeniz University Medical Faculty Hospital, Antalya, Turkey, between May 2006 and July 2007 were included in the study. Ten of them were boys and the mean age at transplantation was 7.97 (5.3). Informed consent was obtained from the parents of children taking part in the study. The Ethics Committee of Akdeniz University, Faculty of Medicine, reviewed and approved the study.

After HSCT, peripheral blood samples were collected from 15 transplant recipients weekly for 2 months and then biweekly for the following month.

Clinical symptoms (fever, rash, CNS dysfunction, pneumonitis, hemorrhagic diarrhea) that might be associated with an active HHV6 infection and presence of acute GVHD were noted. Neutrophil engraftment was defined as an absolute neutrophil count exceeding  $0.5 \times 10^9/L$  for 2 consecutive days, the first day of which was considered the day of engraftment. Platelet engraftment was defined as a platelet count more than  $20 \times 10^9/L$  for 2 consecutive days without platelet transfusion.

Ten of the patients received their grafts from HLA identical siblings, 3 from 1 mismatched unrelated donors, 1 from matched father, and one from matched unrelated donor. Every patient received chemotherapy-based myeloablative conditioning regimen. During the first month after transplantation, patients received cyclosporine A intravenously at 2 mg/(kg.d) as GVHD prophylaxis. Subsequently, it was given orally at 6 mg/(kg.d); acyclovir (100 mg/[kg.d]), fluconazole (5 mg/[kg.d]), and ciprofloxacin (15 mg/[kg.d]) were given orally for 6 months, and trimethoprim sulfamethoxazole (5 mg/[kg.d]) was given orally for a year to all patients. Patients' characteristics are given in **Table 1**.

### Definitions

The presence of HHV6 DNA in plasma was defined as an active HHV6 infection. Fever was defined as a temperature exceeding 38.8°C for at least 3 days without a confirmed etiology.

### Detection and Quantification of HHV6 DNA

DNA extraction was performed manually with DNA extraction kit (Argene SA, Varilhes, France) from 200 µL of plasma. Rotor-Gene 6000 instrument (Corbett Research, Australia) was used with CMV HHV6-8

R-Gene quantification kit (Argene SA, Varilhes, France) according to the manufacturer's instructions for real-time amplification. The threshold of HHV6 detection was 250 copies/mL of whole blood, and none of the samples that were positive for HSV I-II, VZV, JCV, BKV, and Adenovirus 12 gave false positive results according to the package insert.

The presence of PCR inhibitors was evaluated with the internal control amplification that was provided in the kit.

### Risk factors for active HHV6 infection

Sex, age at transplantation, HLA match of graft, active CMV infection, presence of acute GVHD, and use of ATG were evaluated as possible risk factors for active HHV6 infection. For the HLA match of graft, patients were grouped in 2 groups as recipients with HLA identical siblings and others. These variables were also analyzed as risk factors for HHV6 DNA levels exceeding 1000 copies/mL of plasma (**Table 2**).

### Statistical analyses

Statistical tests were performed using SPSS, version 12.01 (SPSS Inc. Chicago, IL, USA). Univariate analyses of variables associated with positive results for HHV6 were performed by chi-square analysis, Fisher exact test, Mann-Whitney U test, and Student *P* test. *P* < .05 was considered to be significant in all analyses.

**Table 1.** Patients' characteristics (n=15).

Age in y (mean/range/SD)	7.97/2-21/5.53
Gender (male/female)	10/5
<b>Underlying disease</b>	
Thalassemia major	7
Acute lymphocytic leukemia	2
Aplastic anemia	2
Griscelli syndrome	1
Acute myeloid leukemia	1
Hurler syndrome	1
Hemophagocytic syndrome	1
<b>Type of donor</b>	
HLA identical sibling	10
One antigen mismatched unrelated	3
Match unrelated	1
Match related	1

SD: Standard deviation.

**Table 2.** Risk factors for HHV6 positivity.

Variables	Total n=15 (%) <sup>a</sup>	HHV6		HHV6 (>1000 copies/ mL) (n=2)	P <sup>c</sup>
		Positive n=8 (%) <sup>b</sup>	Negative n=7 (%) <sup>b</sup>		
Age, y, median	8.0	7.2	8.9	2.0	.27 / .03
Active CMV infection	11 (73%)	6 (55%)	5 (45%)	2	.87 / .24
Male sex	10 (67%)	4 (40%)	6 (60%)	2	.46 / .28
HLA match with donor					
HLA identical siblings	10 (67%)	5 (50%)	5 (50%)	0	.57 / .02
Others	5 (33%)	3 (60%)	2 (40%)	2	
Presence of acute GVHD	2 (13%)	2 (100%)	0 (0%)	2	.09 / .00
Use of ATG	8 (53%)	4 (50%)	4 (50%)	2	.78 / .06

HHV6: Human herpes virus 6, CMV: cytomegalovirus, GVHD: graft versus host disease, ATG: Anti-thymocyte globulin.

<sup>a</sup>Percent values belong to the columns.

<sup>b</sup>Percent values belong to the rows.

<sup>c</sup>P values were determined with 2-tailed Fisher exact test or Mann-Whitney U test. The first P value is for the HHV6 positive and negative groups and the latter one is for the patients having HHV6 positivity higher than 1000 copies/mL and all the other patients.

## RESULTS

### Detection of HHV6 and CMV DNA in plasma

A total of 138 plasma samples from 15 patients were analyzed. The median follow-up for the patients was 84 days (range 46-99) and the samples were collected with a median of 9 samples (range 7-10) per patient. Only 1 of 138 samples was found to have PCR inhibitors, and it was excluded from further analysis. From 137 samples, 21 (15.3%) were positive for HHV6 DNA. The median time to onset of HHV6 viremia was 14 days (range 6-23 days) after transplantation (Figure 1).

HHV6 DNA was found positive in at least 1 sample in 8 of 15 (53%) patients. Four of 8 patients (50%) with active HHV6 infection had only a single positive PCR result. Three patients had consecutive positivities and 1 patient had non-consecutive positivities. The median plasma HHV6 DNA load was 368 copies/mL (range  $4 \times 10^5$ – $2.5 \times 10^5$ ). The levels of HHV6 DNA of the patients with consecutive positivities are shown in Figure 2. For these 3 patients, the onset of infections was 7, 13, and 17 days after transplantation, and while the positivity remained 7 and 14 days for 2 patients, 1 patient was still positive at the end of the study.

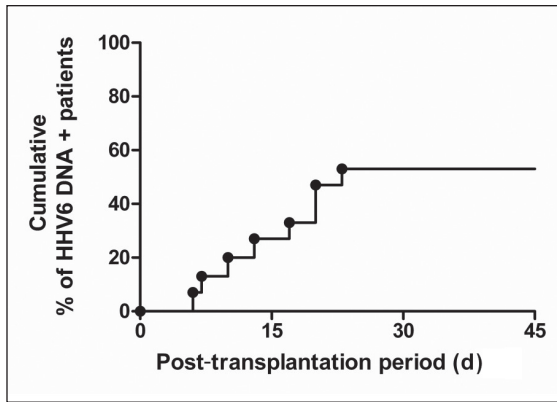
HHV6 DNA levels above 1000 copies/mL were found only in 2 patients, and they were also consecutively positive for HHV6 DNA.

### Risk factors for active HHV6 infection

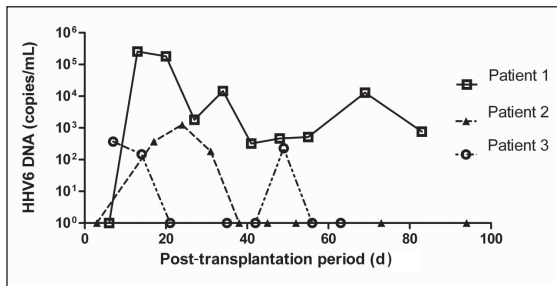
None of the differences for sex, age at transplantation, HLA match of graft, CMV DNA positivity in plasma, presence of acute GVHD, and use of ATG between patients with and without active HHV6 infection was statistically significant. However, when the mean age at transplantation was compared between patients with HHV6 viremia levels higher than 1000 copies/mL and all the other patients, the difference was statistically significant (2 [0], 8.9 [5.1] years old, respectively,  $P=.03$ , Mann-Whitney U,  $Q=0.50$ ). Also presence of acute GVHD and receiving grafts other than HLA identical siblings increased the risk, with a statistically significant difference, of having active HHV6 infection with levels exceeding 1000 copies/mL ( $P=.001$  and  $P=.025$ , respectively, 2-tailed Fisher exact test).

### Relationship of HHV6 DNA with clinical conditions

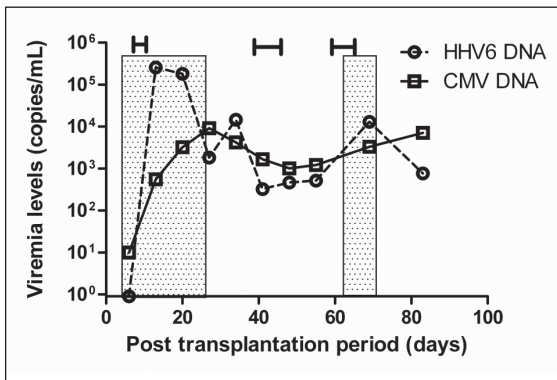
During the follow-up period, 6 patients (40%) developed fever. The mean initiation of fever was at 4.3 (3.0) days (range 2–10) after transplantation, and the mean duration was 10.8 (9.2) days (range 3–23). The two patients who had the longest fever durations were also the only 2 patients with HHV6 viremia levels above 1000 copies/mL. They were also positive for CMV DNA, but their fever were correlated with plasma HHV6 DNA levels (Figure 3). These 2 (13.3%) patients also developed rash, and their rash periods were associated with HHV6 DNA levels. One patient



**Figure 1.** The median time to onset of HHV6 viremia.



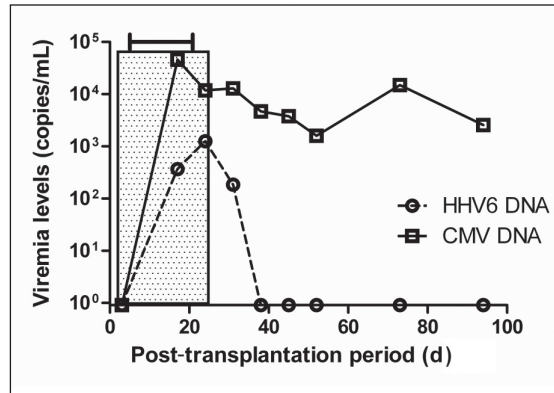
**Figure 2.** Levels of HHV6 DNA of the patients with consecutive positivites.



**Figure 3a.** FUO periods of patient 1 with HHV6 DNA levels above 1000 copies/mL. Shaded areas show the periods of FUO. Capped lines shows the presence of skin rash..

had rash and fever periods that coincided with 2 peaks for HHV6 DNA levels (**Figure 3a**). For the other patient, fever and rash disappeared with the start of the decline of plasma HHV6 DNA level (**Figure 3b**).

Median days to achieve neutrophil and platelet engraftments were 15.9 (range 8-33) and 26.7 (range 13-87) days after transplantation, respectively. This situation was similar for patients infected and non-infected



**Figure 3b.** FUO periods of patient 2 with HHV6 DNA levels above 1000 copies/mL. Shaded areas show the periods of FUO. Capped lines shows the presence of skin rash.

with HHV6. However, the time for platelet engraftment for patients with HHV6 viremia levels higher than 1000 copies/mL (68.5 [26.2] days) was significantly longer when compared with others (20.3 [4.8] days) ( $P=.01$ , Mann Whitney U test).

None of the patients developed any CNS dysfunction.

Acute GVHD was seen in two patients, and these were grade 4 intestinal GHVD. For one patient, acute GVHD developed after HHV6 infection, but for the other patient it was not possible to identify which developed first. These two patients were also the patients having HHV6 viremia levels higher than 1000 copies/mL. The statistical analysis revealed a significant difference for the cooccurrence of acute GVHD and HHV6 viremia levels higher than 1000 copies/mL ( $P=.001$ , 2-tailed Fisher exact test).

In the first year after transplantation, two patients died after the follow-up period; one of them was a case of relapse mortality and the other patient died from cardiac toxicity related with chemotherapy.

## DISCUSSION

This study revealed that active HHV6 infections were common (53%) in pediatric HSCT recipients after transplantation, similar to earlier studies reporting an incidence up to 67%.<sup>12-14</sup> In other studies performed in adult populations, the frequency was moderately lower when compared with pediatric patients.<sup>9-11,15</sup> The reason behind this may be the primary infections that are more frequently encountered in pediatric patients and myeloablative conditionings, which is rarely applied to adult patients. Another reason causing higher frequency of HHV6 viremia in pediatric groups might be the more recently encountered HHV6, resulting in protec-

tive antibodies with lower affinity when compared with adults.<sup>12</sup>

For a viral infection that become latent after primary infections, detecting latent viruses is a potential problem. Kondo et al<sup>7</sup> showed the possibility of latency in PBMCs after active HHV6 infections. Studying HHV6 DNA from plasma samples avoided false positive reactions that could result from latent HHV6 DNA in PBMCs.

The first month after transplantation was found to be the most important time period for the first positivities for HHV6 viremia.<sup>9,12,16</sup> These studies found a median time for the first HHV6 DNA positive samples between 16 and 28 days after transplantation.<sup>9,12,16</sup> In this study the median time to onset of HHV6 viremia was 14 days and the range was between 6 and 24 days after transplantation, which was very consistent with other studies. These findings revealed that the first month after transplantation should be monitored very closely for active HHV6 infections.

Several studies had investigated relationship between sex, age at transplantation, HLA match of graft, active CMV infection, presence of acute GVHD, use of ATG, and active HHV6 infections.<sup>17,18</sup> In this study we could not find any relationship between these variables and active HHV6 infections. But when the analysis was performed with patients having HHV6 DNA levels exceeding 1000 copies/mL of plasma, younger age at transplantation, presence of acute GVHD, and receiving grafts other than HLA identical siblings increased the risk, with a statistically significant difference. Similarly Ljungman et al<sup>19</sup> found significantly higher HHV6 viral loads in patients who received grafts from unrelated stem cell donors or HLA-mismatched family donors than patients who received grafts from matched sibling donors.

Two patients with high-level HHV6 viremia were also the only patients developing acute GVHD and

skin rash. Although the time for skin rash was correlated with HHV6 viremia levels, it was not possible to reveal the cause and effect relation between acute GVHD and HHV6 viremia for one patient. These two highly viremic patients for HHV6 were also the patients with longest fever periods. Another clinical condition found to be related with high HHV6 viremia was prolonged platelet engraftment time. These clinical conditions were also found to be associated with HHV6 viremia in numerous previous studies.<sup>9,10,12,19-22</sup> But only a few of them analyzed high HHV6 viremia levels and clinical outcomes<sup>12,20,21</sup> Ljungman et al<sup>19</sup> and Ogata et al<sup>20</sup> found delayed platelet engraftment in patients with high-level HHV6 viremia. Also a relationship of high-level viremia with acute GVHD was found by de Pagter et al.<sup>12</sup>

Using different primers, probes, and standards for quantitative HHV6 PCR assays can severely limit comparison of studies with each other. With commercial tests using an international quantification standard, comparing results obtained from different laboratories and setting up a cutoff value for clinically significant viremia levels will be much easier.

Approximately, half of pediatric allogeneic HSCT patients develop active HHV6 infection. The presence of acute GVHD, receiving grafts other than HLA identical siblings, or lower age at transplantation seem to be risk factors for developing high viremic loads, which can result in delayed platelet engraftment time, fever, and skin rash. Because of the detection of all active HHV6 infections in the first month after transplantation, the first month after transplantation seems to be very important. For setting up a clinically significant cutoff value for viremia, more studies performed with plasma samples using a commercial real-time quantitative kit are needed. Furthermore, immunological aspects of the virus need to be studied for predicting and controlling active HHV6 infections.

## REFERENCES

1. Cone RW, Huang ML, Ashley R, Corey L. Human herpesvirus 6 DNA in peripheral blood cells and saliva from immunocompetent individuals. *J Clin Microbiol* 1993; 31: 1262–1267.
2. Briggs M, Fox J, Tedder R. Age prevalence of antibody to human herpesvirus 6. *Lancet* 1988; 1: 1058–1059.
3. Okuno T, Takahashi K, Balachandra K, Shiraki K, Yamanishi K, Takahashi M, Baba K. Seroepidemiology of human herpesvirus 6 infection in normal children and adults. *J Clin Microbiol* 1989; 27: 651–653.
4. Rhoads MP, Magaret AS, Zerr DM. Family saliva sharing behaviors and age of human herpesvirus-6B infection. *J Infect* 2007; 54: 623–626.
5. Fox JD, Briggs M, Ward PA, Tedder RS. Human herpesvirus 6 in salivary glands. *Lancet* 1990; 2: 590–593.
6. Eizuru Y, Minematsu T, Minamishima Y, Kikuchi M, Yamanishi K, Takahashi M, Kurata T. Human herpesvirus 6 in lymph nodes. *Lancet* 1989; 1: 40–41.
7. Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K. Latent human herpesvirus 6 infection of human monocytes/macrophages. *J Gen Virol* 1991; 72: 1401–1408.
8. Ljungman P, Urbano-Ispizua A, Cavazzana-Calvo M, Cornelissen J, Witte TD, Dini G, Einsele H, Gaspar HB, Gratwohl A, Passweg J, et al. Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: Definitions and current practice in Europe. *Bone Marrow Transplant* 2006; 37: 439–449.
9. Hentrich MD, Oruzzo G, Jager M, Schlemmer M, Schleuning M, Schiel X, Hiddemann W, Kolb HJ. Impact of human herpesvirus-6 after haematopoietic stem cell transplantation. *Br J Haematol* 2005; 128: 66–72.
10. Zerr DM, Corey L, Kim HW, Huang ML, Nguy L, Boeckh M. Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. *Clin Infect Dis* 2005; 40: 932–940.
11. Yamane A, Mori T, Suzuki S, Mihara A, Yamazaki R, Aisa Y, Nakazato T, Shimizu T, Ikeda Y, Okamoto S. Risk factors for developing human herpesvirus 6 (HHV-6) reactivation after allogeneic hematopoietic stem cell transplantation and its relationship with central nervous system disorders. *Biol Blood Marrow Transplant* 2007; 13: 100–106.
12. de Pagter PJ, Schuurman R, Visscher H, de Vos M, Bierings M, van Loon AM, Uiterwaal CS, van Baarle D, Sanders EA, Boelens J. Human herpes virus 6 plasma DNA positivity after hematopoietic stem cell transplantation in children: an important risk factor for clinical outcome. *Biol Blood Marrow Transplant* 2008; 14: 831–839.
13. Sashihara J, Tanaka-Taya K, Tanaka S, Amo K, Miyagawa H, Hosoi G, Taniguchi T, Fukui T, Kasuga N, Aono T, et al. High incidence of human herpesvirus 6 infection with a high viral load in cord blood stem cell transplant recipients. *Blood* 2002; 100: 2005–2011.
14. Savolainen H, Lautenschlager I, Piiparinen H, Saarinen-Pihkala U, Hovi L, Vetterranta K. Human herpesvirus-6 and -7 in pediatric stem cell transplantation. *Pediatr Blood Cancer* 2005; 45: 820–825.
15. Kadakia MP, Rybka WB, Stewart JA, Patton JL, Stamey FR, Elsayy M, Pellett PE, Armstrong JA. Human Herpesvirus 6: Infection and Disease Following Autologous and Allogeneic Bone Marrow Transplantation. *Blood* 1996; 87: 5341–5354.
16. Schönberger S, Meisel R, Adams O, Pufal Y, Laws HJ, Enczmann J, Dilloo D. Prospective, comprehensive, and effective viral monitoring in children undergoing allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2010; 16: 1428–1435.
17. Jaskula E, Dlubek D, Sedzimirska M, Duda D, Tarnowska A, Lange A. Reactivations of cytomegalovirus, human herpes virus 6, and Epstein-Barr virus differ with respect to risk factors and clinical outcome after hematopoietic stem cell transplantation. *Transplant Proc* 2010; 42: 3273–3276.
18. Yoshikawa T, Asano Y, Ihira M, Suzuki K, Ohashi M, Suga S, Kudo K, Horibe K, Kojima S, Kato K, et al. Human herpesvirus 6 viremia in bone marrow transplant recipients: clinical features and risk factors. *J Infect Dis* 2002; 185: 847–853.
19. Ljungman P, Wang FZ, Clark DA, Emery VC, Remberger M, Ringdén O, Linde A. High levels of human herpesvirus 6 DNA in peripheral blood leucocytes are correlated to platelet engraftment and disease in allogeneic stem cell transplant patients. *Br J Haematol* 2000; 111: 774–781.
20. Ogata M, Kikuchi H, Satou T, Kawano R, Ikewaki J, Kohno K, Kashima K, Ohtsuka E, Kadota J. Human herpesvirus 6 DNA in plasma after allogeneic stem cell transplantation: incidence and clinical significance. *J Infect Dis* 2006; 193: 68–79.
21. Wang LR, Dong LJ, Zhang MJ, Lu DP. The impact of human herpesvirus 6B reactivation on early complications following allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006; 12: 1031–1037.
22. Chevallier P, Hebia-Fellah I, Planche L, Guillaume T, Bressolette-Bodin C, Coste-Burel M, Riolland F, Mohty M, Imbert-Marcille BM. Human herpes virus 6 infection is a hallmark of cord blood transplant in adults and may participate to delayed engraftment: a comparison with matched unrelated donors as stem cell source. *Bone Marrow Transplant* 2010; 45: 1204–1211.