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Human herpesvirus 6 infection after autologous stem cell transplantation: A multicenter prospective study in adult patients

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SUMMARY

Objectives: to prospectively evaluate the incidence and the clinical relevance on hematopoietic reconstitution of HHV-6 infection in autologous hematopoietic stem cell transplantation (ASCT) recipients.
Methods: HHV-6 DNA load was measured in whole blood specimens once during the 7 days before stem cell re-infusion and once a week after transplantation until hematopoietic recovery. Active HHV-6 infection was defined by 2 consecutive positive DNA loads.
Results: from July 2012 to February 2015, 196 adult patients undergoing ASCT were enrolled. Twenty-two (11.2%) patients developed active HHV-6 infection with a cumulative incidence of 19% at 40 days after transplantation. The onset of active HHV-6 infection occurred with a median of 13 days after stem cell re-infusion. HHV-6 infection was associated with an increased frequency of non-infectious complications (OR = 5.05; 95%CI 1.78–14.32; $P < 0.001$). Moreover, the severity of these non-infectious complications was higher in recipients exhibiting HHV-6 infection (OR = 4.62; 95%CI 1.32–16.2; $p < 0.01$). Delayed neutrophils 10 (IQR: 8–14) vs 8 (IQR: 6–11) days and platelets recoveries 15 (IQR: 11.8–18.5) vs 8 (IQR: 4–14) days were observed in patients with active HHV-6 infection compared to non-infected ones.
Conclusions: in this study, 11.2% ASCT recipients presented active HHV-6 infection associated with significantly delayed hematologic reconstitution.

Introduction

Human herpesvirus type 6 (HHV-6) is a widespread roseolovirus which encompasses two different variants: HHV-6A and HHV-6B sharing 75%–95% nucleotide sequence identity. Variant B is the most commonly detected in clinical specimens: it is considered as the causative agent of the *exanthema subitum* childhood disease with an estimated seroprevalence of > 95% after the age of 2 years and of pathologies described in immunocompromised patients.[1–3] To date, variant A seems less frequently detected.[4] Like other herpesviruses, HHV-6 establishes a life-long latency; involved organs are brain, bone marrow and salivary glands, with a strong tropism for T-lymphocytes, hematopoietic CD34+ progenitor cells and microglia.[2,5] HHV-6 is also unique among human viruses because of the ability of both variants for chromosomal integration (ci-HHV-6).[6]

If only few cases of HHV-6 symptomatic reactivation have been reported in immunocompetent patients,[7] HHV-6 reactivation rather occurs in immunocompromised hosts such as allogeneic hematopoietic stem cell transplantation (allo-SCT) recipients,[8,9] solid organ transplanted patients,[10] and HIV-infected patients,[11] causing diverse benign to severe clinical manifestations including fever,[12] thrombocytopenia, encephalitis,[13] pneumonitis and hepatitis.[14] In the allo-SCT setting, HHV-6 opportunistic infection

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is associated with poor outcome, including acute graft-versus-host disease (GVHD),[15,16] susceptibility to cytomegalovirus (CMV) disease,[17] and delayed platelet recovery[18] resulting in an increased transplant related mortality.[19]

Autologous hematopoietic stem cell transplantation (ASCT) is widely used for the treatment of myeloma and lymphoma[20] as well as some solid tumours.[21] ASCT patients are generally thought to have less viral infections than allo-SCT patients and, apart systematic CMV viraemia measurement, other herpesviruses are not regularly monitored in ASCT patients. Nevertheless, some ASCT recipients may develop delayed haematological recovery[8,22] but also fever,[23] febrile neutropenia, thrombocytopenia, microangiopathy, diarrhoea, interstitial pneumonitis, encephalitis and cutaneous rashes,[24] all of them being compatible with HHV-6 infection.[25] To date, the number of studies exploring viral infections in ASCT population is limited with only small series, evaluating the impact of HHV-6 infection in paediatric ASCT,[26,27] mixing the analysis of allo-SCT and ASCT,[9] or using mostly qualitative assays without measurement of the viral load; no recent data with clear recommendations for the follow-up of HHV-6 infection in adult ASCT is published.

The primary objective of this prospective multicentre non-randomized study was to evaluate the incidence of HHV-6 infections in adult ASCT recipients using a strict definition of active HHV-6 infection by 2 consecutive DNA loads measured by quantitative real-time PCR (qPCR) in whole blood specimens. Secondary endpoints included the clinical consequences of this infection on hematopoietic reconstitution, CMV co-infection and other infectious and non-infectious complication.

Patients and methods

Patients and study design

Adult patients, undergoing ASCT regardless of haematological malignancies at Saint-Etienne, Lyon and Clermont-Ferrand University Hospitals in France, were prospectively enrolled in this longitudinal multicentre non-randomized VIRAUTO6 study (ClinicalTrials.gov NCT02090803) between July 2012 and February 2015. Patients already included in the present study and receiving a second auto graft were excluded. Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki. The study was approved by the local Ethics Committee of Saint-Etienne and was established for the unique purpose of studying HHV6.

The follow-up period started at stem cell re-infusion day and ended at hospital discharge if hematopoietic recovery was reached without transfusion support, and with a maximum of 40 days after transplantation. Chemotherapy-related toxicities were assessed according to the common terminology criteria for adverse events (CTCAE) classification.[28]

HHV-6 and CMV DNA monitoring

Whole blood quantitative HHV-6 DNA was measured once during the 7 days before stem cell re-infusion and once per week after transplantation, until hematopoietic reconstitution. The test was centralized in the Laboratory of infectious Agents and Hygiene of Saint-Etienne University Hospital. After sampling, whole blood was immediately frozen at −20 °C and sent on the same day to the laboratory. HHV-6 DNA load was measured by qPCR in whole blood specimens as previously described.[29] The limit of quantification was estimated to 450 copies/mL. HHV-6 DNA from both variants (HHV6-A and HHV6-B) was amplified by the assay with consensus primers without differentiation.

Because HHV-6 is frequently associated with CMV infection,[12,22] CMV monitoring was performed in parallel; CMV DNA loads were quantified in the virology laboratory of each participating centre by using their own qPCR-based CMV commercial kit on whole blood specimens sampled the same day as that for HHV-6 DNA load determination.

Definitions

In order to exclude very low and transient HHV-6 DNA loads, active HHV-6 infection was defined as 2 consecutive blood HHV-6 DNAemias $\geq$ 450 copies/mL, one week apart. In case of viral load$>$ 100,000 copies/mL on 2 samples, ci-HHV6 was suspected and a piece of dander (finger nail or hair follicle) was analysed for HHV-6 DNA load; ci-HHV-6 was assessed if HHV-6 DNA was detected in dander (finger nail or hair follicle).

BEAM conditioning regimen includes Carmustine, Etoposide, Cytarabine and Melphalan chemotherapies.

Neutropenia recovery was defined as absolute neutrophil count (ANC) $> 0.5 \times 10^9$/L for two consecutive days. Platelets recovery was defined as platelets count $> 20 \times 10^9$/L without transfusion support. The neutropenia and thrombocytopenia periods were defined as the time from stem cell re-infusion to neutrophils and platelets recoveries without transfusion support, respectively.

HHV-6 clinical disease in our cohort was defined according to the combination of the following criteria as previously reported:[12] the convergence between the chronology of clinical events and the dynamics of HHV-6 DNAemia, the correspondence between the nature of symptoms and the bodily site of HHV-6 infection and the absence of any other pathogen known as cause of the disease.

Statistical analyses

Before starting the study, a sample size of 196 inclusions was planned to give a HHV-6 reactivation cumulative incidence of 48%[30] with a confidence interval of 95% and an accuracy of 7%. All quantitative data were expressed as median with interquartile range (IQR). All categorical data were expressed as frequencies (percent). Quantitative data were compared between groups using the Kruskall–Wallis test; categorical data were compared using the χ^2-test (or the Fisher exact test). The incidence analyses and related figures were performed using the Kaplan–Meyer method (Log-rank test). To analyse the association between HHV6 reactivation and neutrophils/platelets recoveries, a landmark analysis was performed including as landmark time the median of reactivation (13 days). Cox models were used for multivariate survival analyses. Only variables with p-value < 0.2 in univariate analysis were introduced in the multivariate models.

Statistical analyses were carried out using R software version 3.2.5. All P values were two-sided, with $P < 0.05$ denoting statistical significance.

Results

Patients' characteristics

Between July 2012 and February 2015, 196 adult patients underwent peripheral blood ASCT and were included in our study. The patient characteristics are summarised in Table 1. The median follow-up was 16 (IQR: 14-20) days. No patients died over the whole follow-up period.

Incidence of active HHV-6 infection and HHV-6 clinical disease

Twenty-two patients (11.2%) developed an active HHV-6 infection as defined above, with a cumulative incidence of 19% at 40

Table 1
Baseline characteristics of the patients according to HHV-6 infected status.

Patients' baseline characteristics	Non-infected patients ($n = 174$) n (%)	HHV-6-infected patients ($n = 22$) n (%)	Total ($n = 196$) n (%)	P-value
Sex (male/female)	113/61	14/8	127/69	0.99
Median age (range)	59.5 (53.6 - 64.8)	58.2 (48.6 - 61.8)	59.4 (52.5-64.8)	0.29
Underlying diseases				
Non Hodgkin lymphoma	72 (41.4%)	17 (77.3%)	89 (45.4%)	0.001
Multiple myeloma	84 (48.3%)	2 (9.1%)	86 (43.9%)	
Hodgkin lymphoma	16 (9.2%)	3 (13.6%)	19 (9.7%)	
Acute leukemia	1 (0.6%)	0	1 (0.5%)	
NA	1 (0.6%)	0	1 (0.5%)	
Non Hodgkin Lymphoma				
Diffuse large B cell lymphoma	32(44.4%)	8 (47.1%)	40 (44.9%)	0.72
Mantle cell lymphoma	14 (19.4%)	4 (23.5%)	18 (20.2%)	
Follicular lymphomas	11 (15.3%)	4 (23.5%)	15 (16.9%)	
T cell lymphomas	8 (11.1%)	1 (5.9%)	9 (10.1%)	
Others	7 (9.7%)	0	7 (7.9%)	
Disease status at transplantation				
CR and VGPR	111 (63.8%)	14 (63.6%)	125 (63.8%)	0.99
PR/SD	62 (35.6%)	8 (36.4%)	70 (35.7%)	
RD	1 (0.6%)	0	1 (0.5%)	
Conditioning regimen				
BEAM	76 (43.7%)	17 (77.3%)	93 (47.4%)	< 0.001
Melphalan	85 (48.9%)	2 (9.1%)	87 (44.4%)	
Others	13 (7.5%)	3 (13.6%)	16 (8.2%)	
Number of treatment prior to transplantation				
1	102 (58.6%)	12 (54.5%)	114 (58.2%)	0.82
2-4	72 (41.4%)	10 (45.5%)	82 (41.8%)	
CMV serostatut				
CMV -	88 (53.3%)	14 (70%)	102 (55.1%)	0.23
CMV +	77 (46.7%)	6 (30%)	83 (44.9%)	
NA	9 (5.2%)	2 (9.1%)	11 (5.6%)	

NA = Not available, CR = Complete remission, VGPR = very good partial response, PR = partial response, SD = stable disease, RD = refractory disease.
BEAM = Carmustine-Etoposide-Cytarabine-Melphalan conditioning regimen; CMV - =Negative serostatus for cytomegalovirus (CMV); CMV + = Positive serostatus for CMV.

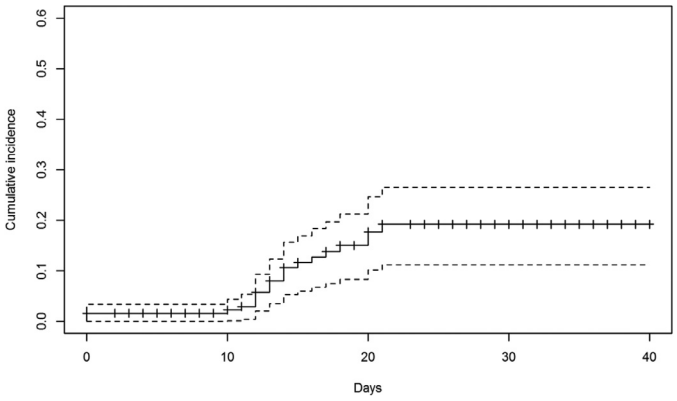

Fig. 1. HHV-6 infection cumulative incidence. Dotted lines represent standard deviations. HHV-6 infection occurred with a cumulative incidence of 19% at 40 days after transplantation.

days after transplantation (Fig. 1). Fifty-eight patients exhibited a positive (≥ 450 copies/mL) HHV-6 DNA load on a single whole blood specimen with 30% of incidence. However, these additional cases were not considered as active HHV-6 infections according to our definition and they were included into the control group (i.e. non-infected patients). HHV6 was not detected before ASCT except for 3 patients who exhibited a very low HHV-6 DNAemia (just equal to 450 copies/mL) before stem cell re-infusion; all these 3 patients developed HHV-6 infection after ASCT. Among the 196 patients, none was suspected for ci-HHV-6 and no case was described.

Active HHV-6 infection occurred with a median of 13 days (IQR: 12-15.8) after transplantation and a median blood HHV-6 DNAemia of 7035 copies/mL (IQR: 1192.8–19,875.7). Among the patient's characteristics (Table 1), only the underlying diseases and the conditioning regimen differed significantly between the 2 groups with more BEAM regimen and lymphoma in the group with active HHV-6 infected patients. In univariate analysis, neither sex, age, disease status at time of stem cell re-infusion, number of courses of chemotherapy preceding ASCT nor the conditioning regimen did favour HHV-6 infection (Table 2). Nevertheless, multivariate survival analysis could not be performed since no variable had a p-value <0.2 in univariate analysis.

For 3 patients, symptoms were compatible with HHV-6 clinical disease (1.5% of the cohort): 2 patients had skin rash with positive skin biopsy for HHV-6 DNA and 1 patient had fever with no other cause than HHV-6 infection. For 2 patients, ganciclovir treatment was introduced successfully for a median duration of 12 (Range: 8-15) days.

Active HHV-6 infection and CMV co-infection

Only one active CMV infection with at least 2 positives consecutive CMV DNA loads during the same period was observed in a 69-year-old man undergoing BEAM-ASCT for a mantle-cell lymphoma. At transplant time, his haematological disease was in partial response. He suffered from grade 3 mucositis. Neutropenia recovery took 12 days while platelets recovery took 30 days. The HHV-6 DNA load was positive 22 days after ASCT, with a value of 10,900 copies/mL and a persistent HHV-6 DNA load was observed the 18 following days, at a lower value however (between 636 and 1450 copies/mL). At day 27 post-ASCT, a positive CMV DNA load was detected (1050 copies/mL, i.e. 231 UI/mL) and persisted until the end of the 40 days follow-up for this patient. This patient did not receive any antiviral treatment.

Active HHV-6 infection and hematopoietic reconstitution

During the study period, all patients recovered from neutropenia, and 173 patients (88.3%) recovered from thrombocytopenia.

Table 2
Univariate analysis of HHV-6 reactivation with the survival analysis method (HHV-6 taken as a time-dependent variable).

		HR.95.CI.	P.Wald.s.test.	P-value
Sex	Male vs Female	1.18 (0.86,1.62)	0.306	0.311
Age	ref.=(18,52]			0.847
	(52,60]	0.98 (0.64,1.49)	0.919	
	(60,65]	0.97 (0.62,1.53)	0.912	
	(65,71]	1.14 (0.73,1.77)	0.574	
Disease status at transplantation	PR+SD+RD vs CR and VGPR	0.94 (0.68,1.29)	0.686	0.715
Conditioning regimen	ref.=BEAM			0.055
	Other	1.6 (0.88,2.89)	0.123	
	melphalan	1.42 (1.03,1.94)	0.03	
Number of treatment prior to transplantation	ref.=1			0.41
	2	1.04 (0.75,1.45)	0.819	
	3	1.05 (0.56,1.98)	0.867	
	4	0.51 (0.22,1.18)	0.116	

CR = Complete remission, VGPR = very good partial response, PR = partial response, SD = stable disease, RD = refractory disease.
BEAM = Carmustine-Etoposide-Cytarabine-Melphalan conditioning regimen.

Table 3
Comparison of non-infectious with infectious complications according to HHV-6 infected status.

$N = 196$	Non-infected patients n (%)	HHV-6-infected patients n (%)	Total n (%)
Non infectious complications			
Oral mucositis	141 (81%)	17 (77.3%)	158 (80.8%)
Grade 1	33 (23.4%)	2 (11.8%)	35 (22.2%)
Grade 2	38 (27%)	1 (5.9%)	39 (24.7%)
Grade 3	52 (36.9%)	12 (70.6%)	64 (40.5%)
Grade 4	15 (10.6%)	2 (11.8%)	17 (10.8%)
NA	3 (2.1%)	0 (0%)	3 (1.9%)
Diarrhea	127 (73%)	9 (40.9%)	136 (69.4%)
Liver enzyme elevation	111 (63.8%)	16 (72.7%)	127 (64.8%)
Skin rash	58 (33.3%)	11 (50%)	69 (35.2%)
Acute kidney injury	23 (13.2%)	4 (18.2%)	27 (13.8%)
Infectious complications			
Febrile neutropenia	109 (62.6%)	15 (68.2%)	124 (63.3%)
Clinically/microbiologically sites involved			
Gut	50 (28.7%)	5 (22.7%)	55 (28.1%)
Urinary tract	49 (28.2%)	7 (31.8%)	56 (28.6%)
Septicaemia	53 (30.5%)	8 (36.4%)	61 (31.1%)
Lung	10 (5.7%)	1 (4.5%)	11 (5.6%)

The median neutropenia and thrombocytopenia durations were 8 (IQR: 7-11) days and 8 (IQR: 4-16) days, respectively. Delayed ANC and platelets recoveries were observed in patients with active HHV-6 infection compared to those without HHV-6 infection. The median duration of ANC recovery was increased to 10 (IQR: 8 - 14) vs 8 (IQR: 6-11) days. Recipients exhibiting active HHV-6 infection had platelets recovery duration longer whatever the threshold used: platelets recovery $> 20 \times 10^9$/L, 15 vs 8 days and platelets recovery $> 50 \times 10^9$/L, 25 *vs* 15 days. (cf. Fig. 2A and 2B).

Therefore, the duration of hospitalisation was significantly longer for patients with active HHV-6 infection with a median duration of 30.5 days (IQR: 26.2 - 34) vs 22 days (IQR: 19 - 25) for patients without infection ($P < 0.001$). Similarly, HHV-6-infected patients required transfusions later than non-infected patients: the median time between transplant and last transfusion was longer for HHV-6-infected patients with 17 days (IQR: 15–22) compared to those without infection with 12.5 days (IQR: 10 - 18; $P = 0.006$).

Active HHV-6 infection and other complications

Febrile neutropenia occurred in 124 patients (63.3%); the main site of infection was peripheral blood (septicaemia, 31.1%) and the main pathogens identified were Gram negative bacilli (24.3%), (Table 3). For one third of patients (33.4%), febrile neutropenia was not documented.

Non-infectious complications occurred in 195 patients (99.5%) with a median of 4 complications (IQR: 3-5) per patient. These complications were grade 1 and 2 in 68.4% of cases, grade 3 in 23.3% of cases and grade 4 in 3.4% of cases. The most frequent non-infectious complication was oral mucositis that occurred in 158 patients (80.6%) with a maximum grade 3 in 40.5% of cases. The mucositis median duration was 8 days (IQR: 5-11). The other frequent non-infectious complications were diarrhoea (69.4%), liver enzyme elevation (64.8%), skin rash (35.2%) and acute kidney injury (13.8%).

Although diarrhoea and mucositis were more frequent in non HHV-6 infected patients than in infected ones (Table 3), active HHV-6 infection was associated with an increased number of combined non-infectious complications (OR 5.05; 95%CI 1.78-14.32; $P < 0.001$). Moreover, the severity of these complications was higher in this group with more grade 3-4 complications (0R 4.62; 95%CI 1.32-16.2; $P = 0.006$).

Discussion

To date, this study is the first large-scaled multicentre prospective non-randomized study including 196 autologous stem cell transplants recipients. The first aim was to determine the incidence of active HHV-6 infection: it was of 11.2% with a cumulative incidence of 19% at 40 days after transplantation. Few studies had already addressed this question in the setting of ASCT: the retrospective works of Imbert-Macille et al., Inazawa et al. and more recently Colombier et al. reported an incidence of HHV-6 infection of 42.5%, 11.4%, and 8;5%, respectively.[9,23,31] All defined the presence of any level of HHV-6 DNA in blood as active HHV-6

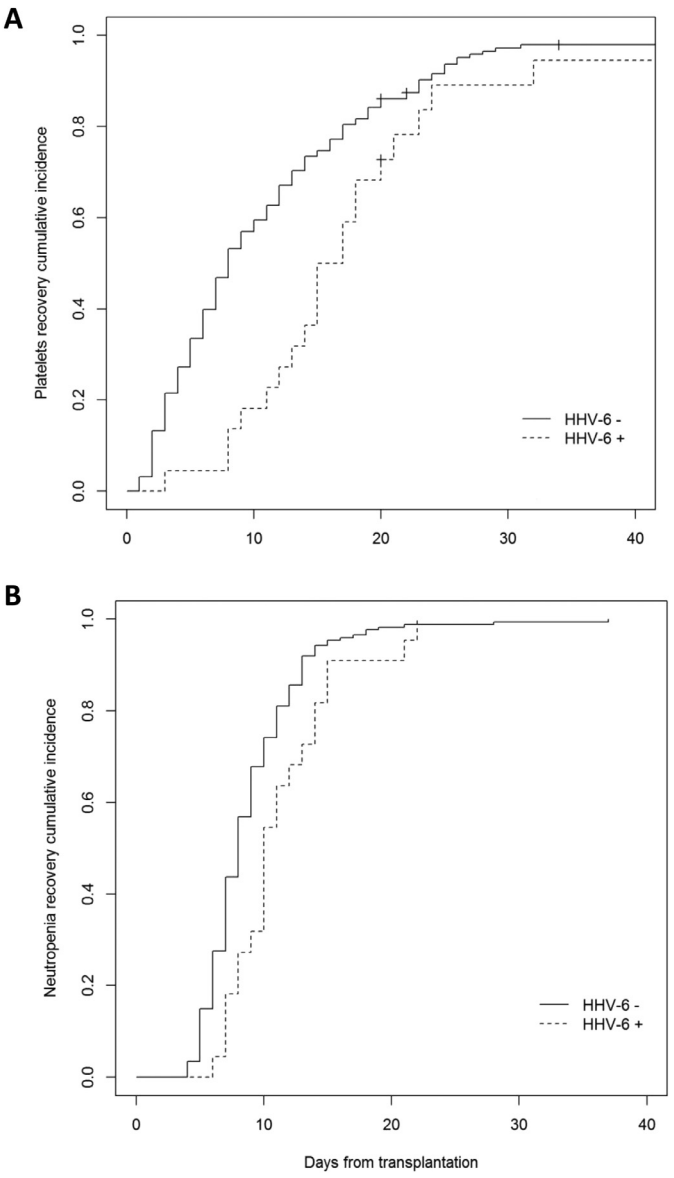

Fig. 2. A; Kinetics of platelet recovery (platelets $> 20 \times 10^9$/L) according to HHV-6 infection. HHV-6 +: recipients exhibiting HHV-6 infection; HHV-6 -: recipients without HHV-6 infection.
B; Kinetics of neutropenia recovery (ANC $> 0.5 \times 10^9$/L) according to HHV-6 infection. HHV-6 +: recipients exhibiting HHV-6 infection; HHV-6 -: recipients without HHV-6 infection.

infection. In our work, in order to overcome blips of DNAemia, 2 consecutive blood HHV-6 DNAemias were needed to assess the diagnosis of active HHV-6 infection and to appreciate its kinetics. Up to date, no threshold has been formally recognised as the frontier between latent infection and active infection: in order not to omit low reactivations, we opted for a reference value of blood HHV-6 DNAemias $\geq$ 450 copies/mL. By using the same criteria as in the studies listed just above, the HHV-6 incidence raised to almost 30% in our study. In accordance with Imbert-Marcille et al. who assessed that active HHV-6 infection frequently occurred early after transplantation with a median of 16 days in the ASCT cohort,[9] HHV-6 infection occurred with a median time of 13 days in our cohort.

HHV-6 and CMV DNAemias are either monitored on whole blood or plasma specimen, depending on the choice of the laboratory. In Europe, and especially in France,[4,22,23,29,32,33] whole blood is the first used, mainly because this specimen has very limited preparation phases at the preanalytical step (no isolation of leucocytes and no centrifugation of plasma that could lyse cells[1]). This specimen can also be used in case of agranulocytosis, allows the detection of virus replication earlier, and allows the detection of the ci-HHV-6 when present.[4,6] Most studies cited above monitored HHV-6 in whole blood specimen.[23,31,34] Although detection of viral mRNA could be useful to analyse latent,[35] this tool is not currently used in routine and consequently we could not conclude on the presence of latent infection in our patients.

Moreover, given the HHV-6 DNA loads were all $<$ 100,000 copies/mL and not persistent, we could exclude ci-HHV-6. As the patients were all adults, the probability they had already met the virus was high. Consequently, we considered that active HHV-6 infections were reactivations.

The main limitation of our study is that the median follow-up of patients was shorter (16 days) than expected initially (ideally 40 days), which could have led to miss a few delayed infections and participate to minimize HHV-6 incidence. This short follow-up does not result neither from an early and voluntary study exit decided by clinicians nor a lost to follow-up, but it is rather explained by hospital discharge at the time of neutropenia recovery whatever platelet recovery or transfusion support need.

In patients with haematological malignancies and after stem cell transplantation, Ljungman et al. defined HHV-6 infection as HHV-6 detected in a previously HHV-6-seropositive patient.[36] In our cohort, clinical relevance of HHV-6 infection was low, as it has been already reported in ASCT patients.[31] However, HHV-6 disease could be highly suspected for 3 patients, with detection of HHV-6 DNA in skin biopsy for 2 of them. This was also reported in the literature.[34]

HHV-6 infection occurred more frequently in patients with BEAM conditioning regimen. However, BEAM is more used for lymphoma in which immunity was probably lower than plasmocytoma disorder in part because of immunotherapy as rituximab used before. Moreover, due to supply difficulties of Carmustine, some patients ($n = 16$) received Bendamustine, an immunosuppressive agent combining alkylating and antimetabolite properties known to cause T-cell lymphopenia.[37] By now, it is too early to assess whether this regimen (Bendamustime-EAM) promoted viral infections but vigilance regarding this question is required in the future.

We also hypothesized that HHV-6 infections may correlate with other opportunistic challenging viruses such as CMV. Both CMV and HHV-6 are lymphotropic viruses and are reported to be simultaneously or successively detected in allo-SCT recipients.[12,22] In our cohort, only one patient had concomitant CMV and HHV-6 infections. This low association is concordant with previous studies: Jeulin et al. showed that HHV-6 DNAemia was not significantly associated with CMV infection in a cohort of 220 allo-SCT patients including 44 HHV-6 infections;[32] Horowitz et al. also showed only one patient with concurrent reactivation of CMV out of the 10 ASCT patients diagnosed with HHV-6 reactivation.[38]

The second objective of our study was to analyse hematopoietic reconstitution in ASCT patients. $CD34^+$hematopoietic progenitors can indeed carry latent HHV-6 and hematopoietic differentiation can lead to HHV-6 reactivation giving an explanation for myelosuppression.[39] In allo-SCT recipients, presence of HHV-6 DNA was significantly associated with delayed platelet and neutrophil engraftment.[8,22] In our cohort of ASCT patients, we observed a delay in platelets and ANC reconstitution with consequences on durations of hospitalisations and need of late transfusions in patients with HHV-6 infections, potentially increasing the costs. One tricky point is that this delayed hematopoietic reconstitution occurred before HHV-6 reactivation. However, it is difficult to precisely date the onset time of HHV-6 infection in clinical practice: as our definition of HHV-6 infection was very stringent and took 7 days, HHV-6 could have clinical consequences even at infra-biological thresholds

as seen with CMV,[40] or at the moment of the virus reactivation during the week apart between the 2 measurements. This is one of the explanations of the occurrence of delayed neutropenia recovery prior to the median of onset time of HHV-6 infection: neutropenia recovery is delayed by 2 days (10 versus 8 days) during HHV-6 infection while the median of onset time of HHV-6 infection is 13 days.

Furthermore, infected HHV-6 patients of our series exhibited more frequent and more severe non-infectious complications such as oral mucositis than those without HHV-6 infection. Although this data could be partly biased because HHV-6 infection was more frequent in case of BEAM conditioning regimen, and because it is difficult to precisely date the onset time of a complication, there is a continuum between the beginning and the paroxysm of the complication especially for the mucositis. Actually, this VIRAUTO6 study was not designed to follow each complication in time and to use each variable as the primary endpoint. Our objective was mainly to make a descriptive study concerning HHV-6.

In conclusion, although systematic monitoring of HHV-6 DNAemia could not be recommended for all patients, HHV-6 infection must be evocated in case of delayed hematopoietic reconstitution or severe acute combined toxicities, notably after lymphoma's regimen. This study marks a step forward, but larger studies with patients receiving the same conditioning regimen prior to stem cell reinfusion would be warranted.

Conflict of interest statement

There are no conflicts of interest to report.

Authorship statement

Marie Balsat and Jérôme Cornillon conceived the study, provided clinical care, recorded and collected clinical data, analysed data, and wrote the manuscript.

Sylvie Pillet performed biological analyses, recorded and collected biological data and wrote the manuscript.

Mathieu Oriol and Véronique Bousser performed statistical analyses and commented on the manuscript.

Emmanuelle Tavernier, Victoria Cacheux, Cécile Moluçon-Chabrot and Karine Augeul-Meunier provided clinical care, recorded clinical data and commented on the manuscript.

Vanessa Escuret, Audrey Mirand and Christel Regagnon performed biological analyses.

Fabien Tinquaut performed statistical analyses.

Gilles Salles and Bruno Pozzetto wrote and revised the manuscript.

Jacques-Olivier Bay and Denis Guyotat commented on the manuscript.

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