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► To cite this version:

Marie Balsat, Sylvie Pillet, Emmanuelle Tavernier, Victoria Cacheux, Vanessa Escuret, et al.. Human herpesvirus 6 infection after autologous stem cell transplantation: A multicenter prospective study in adult patients. *Journal of Infection*, 2019, 79 (1), pp.36-42. 10.1016/j.jinf.2019.05.001 . hal-02299171

HAL Id: hal-02299171

<https://hal.science/hal-02299171>

Submitted on 25 Oct 2021

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1 **Human Herpesvirus 6 Infection after Autologous Stem Cell Transplantation: A**
2 **Multicenter Prospective Study in Adult Patients.**

3
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33

34 **Keywords:** HHV-6, autologous stem cell transplantation, early infection,
35 thrombocytopenia, neutropenia

36

37 **Running title:** HHV-6 after Autologous Stem Cell Transplantation

38

39 **ABSTRACT**

40

41 **Objectives:** to prospectively evaluate the incidence and the clinical relevance on
42 hematopoietic reconstitution of HHV-6 infection in autologous hematopoietic stem cell
43 transplantation (ASCT) recipients.

44 **Methods:** HHV-6 DNA load was measured in whole blood specimens once during the 7
45 days before stem cell re-infusion and once a week after transplantation until
46 hematopoietic recovery. Active HHV-6 infection was defined by 2 consecutive positive
47 DNA loads.

48 **Results:** from July 2012 to February 2015, 196 adult patients undergoing ASCT were
49 enrolled. Twenty-two (11.2%) patients developed active HHV-6 infection with a
50 cumulative incidence of 19% at 40 days after transplantation. The onset of active HHV-6
51 infection occurred with a median of 13 days after stem cell re-infusion. HHV-6 infection
52 was associated with an increased frequency of non-infectious complications (OR = 5.05;
53 95%CI 1.78-14.32; $P < .001$). Moreover, the severity of these non-infectious
54 complications was higher in recipients exhibiting HHV-6 infection (OR = 4.62; 95%CI
55 1.32-16.2; $p < .01$). Delayed neutrophils 10 (IQR: 8-14) vs 8 (IQR: 6-11) days and platelets
56 recoveries 15 (IQR: 11.8-18.5) vs 8 (IQR: 4-14) days were observed in patients with
57 active HHV-6 infection compared to non-infected ones.

58 **Conclusions:** in this study, 11.2% ASCT recipients presented active HHV-6 infection
59 associated with significantly delayed hematologic reconstitution.

60 Words: 199

61

62 **Highlights:**

63 -Active HHV-6 infection occurred in 11.2% of autologous stem cell recipients

64 -HHV-6 infection is associated to increased frequency of non-infectious complications

- 65 -Non-infectious complications are more severe when associated to HHV-6 infection
- 66 - HHV-6 infection is associated with delayed neutrophils and platelets recoveries

67 INTRODUCTION

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

79 If only few cases of HHV-6 symptomatic reactivation have been reported in
80 immunocompetent patients(7), HHV-6 reactivation rather occurs in
81 immunocompromised hosts such as allogeneic hematopoietic stem cell transplantation
82 (allo-SCT) recipients(8, 9) , solid organ transplanted patients(10) and HIV-infected
83 patients(11), causing diverse benign to severe clinical manifestations including
84 fever(12), thrombocytopenia, encephalitis(13), pneumonitis and hepatitis(14). In the
85 allo-SCT setting, HHV-6 opportunistic infection is associated with poor outcome,
86 including acute graft-versus-host disease (GVHD)(15, 16), susceptibility to
87 cytomegalovirus (CMV) disease(17), and delayed platelet recovery(18) resulting in an
88 increased transplant related mortality(19).

89 Autologous hematopoietic stem cell transplantation (ASCT) is widely used for the
90 treatment of myeloma and lymphoma(20) as well as some solid tumours(21). ASCT
91 patients are generally thought to have less viral infections than allo-SCT patients and,

92 apart systematic CMV viraemia measurement, other herpesviruses are not regularly
93 monitored in ASCT patients. Nevertheless, some ASCT recipients may develop delayed
94 haematological recovery(8, 22) but also fever(23), febrile neutropenia,
95 thrombocytopenia, microangiopathy, diarrhoea, interstitial pneumonitis, encephalitis
96 and cutaneous rashes(24), all of them being compatible with HHV-6 infection(25). To
97 date, the number of studies exploring viral infections in ASCT population is limited with
98 only small series, evaluating the impact of HHV-6 infection in paediatric ASCT(26, 27),
99 mixing the analysis of allo-SCT and ASCT(9), or using mostly qualitative assays without
100 measurement of the viral load; no recent data with clear recommendations for the
101 follow-up of HHV-6 infection in adult ASCT is published.

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

108 **PATIENTS AND METHODS**

109

110 **Patients and Study Design**

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

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

124

125 **HHV-6 and CMV DNA Monitoring**

126 Whole blood quantitative HHV-6 DNA was measured once during the 7 days before stem
127 cell re-infusion and once per week after transplantation, until hematopoietic
128 reconstitution. The test was centralized in the Laboratory of infectious Agents and
129 Hygiene of Saint-Etienne University Hospital. After sampling, whole blood was
130 immediately frozen at -20°C and sent on the same day to the laboratory. HHV-6 DNA
131 load was measured by qPCR in whole blood specimens as previously described(29). The
132 limit of quantification was estimated to 450 copies/mL. HHV-6 DNA from both variants

133 (HHV6-A and HHV6-B) was amplified by the assay with consensus primers without
134 differentiation.

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

139

140 **Definitions**

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

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

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

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

158 **Statistical Analyses**

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

171 Statistical analyses were carried out using R software version 3.2.5. All *P* values were
172 two-sided, with *P* <.05 denoting statistical significance.

173 **RESULTS**

174

175 **Patients' characteristics**

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

180

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

182 Twenty-two patients (11.2%) developed an active HHV-6 infection as defined above,
183 with a cumulative incidence of 19% at 40 days after transplantation (Figure 1). Fifty-
184 eight patients exhibited a positive (≥ 450 copies/mL) HHV-6 DNA load on a single whole
185 blood specimen with 30% of incidence. However, these additional cases were not
186 considered as active HHV-6 infections according to our definition and they were
187 included into the control group (*i.e* non-infected patients). HHV6 was not detected
188 before ASCT except for 3 patients who exhibited a very low HHV-6 DNAemia (just equal
189 to 450 copies/mL) before stem cell re-infusion; all these 3 patients developed HHV-6
190 infection after ASCT. Among the 196 patients, none was suspected for ci-HHV-6 and no
191 case was described.

192 Active HHV-6 infection occurred with a median of 13 days (IQR: 12-15.8) after
193 transplantation and a median blood HHV-6 DNAemia of 7035 copies/mL (IQR: 1192.8 –
194 19875.7). Among the patient's characteristics (Table 1), only the underlying diseases
195 and the conditioning regimen differed significantly between the 2 groups with more
196 BEAM regimen and lymphoma in the group with active HHV-6 infected patients. In
197 univariate analysis, neither sex, age, disease status at time of stem cell re-infusion,

198 number of courses of chemotherapy preceding ASCT nor the conditioning regimen did
199 favour HHV-6 infection (Table 2). Nevertheless, multivariate survival analysis could not
200 be performed since no variable had a p-value <0.2 in univariate analysis.

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

205

206 **Active HHV-6 infection and CMV co-infection**

207 Only one active CMV infection with at least 2 positives consecutive CMV DNA loads
208 during the same period **was observed in** a 69-year-old man undergoing BEAM-ASCT for
209 a mantle-cell lymphoma. At transplant time, his haematological disease was in partial
210 response. He suffered from grade 3 mucositis. Neutropenia recovery took 12 days while
211 platelets recovery took 30 days. The HHV-6 DNA load was positive 22 days after ASCT,
212 with a value of 10,900 copies/mL and a persistent HHV-6 DNA load was observed the 18
213 following days, at a lower value however (between 636 and 1,450 copies/mL). At day 27
214 post-ASCT, a positive CMV DNA load was detected (1,050 copies/mL, i.e. 231 UI/mL)
215 and persisted until the end of the 40 days follow-up for this patient. This patient did not
216 receive any antiviral treatment.

217

218 **Active HHV-6 infection and hematopoietic reconstitution**

219 During the study period, all patients recovered from neutropenia, and 173 patients
220 (88.3%) recovered from thrombocytopenia. The median neutropenia and
221 thrombocytopenia durations were 8 (IQR: 7-11) days and 8 (IQR: 4-16) days,
222 respectively. Delayed **ANC** and platelets recoveries were observed in patients with

223 active HHV-6 infection compared to those without HHV-6 infection. The median
224 duration of ANC recovery was increased to 10 (IQR: 8 - 14) vs 8 (IQR: 6-11) days.
225 Recipients exhibiting active HHV-6 infection had platelets recovery duration longer
226 whatever the threshold used: platelets recovery > 20 x 10⁹/L, 15 vs 8 days and platelets
227 recovery > 50 x 10⁹/L, 25 vs 15 days. (cf. Figures 2A and 2B).

228 Therefore, the duration of hospitalisation was significantly longer for patients with
229 active HHV-6 infection with a median duration of 30.5 days (IQR: 26.2 - 34) vs 22 days
230 (IQR: 19 - 25) for patients without infection (*P* <0.001). Similarly, HHV-6-infected
231 patients required transfusions later than non-infected patients: the median time
232 between transplant and last transfusion was longer for HHV-6-infected patients with 17
233 days (IQR: 15 - 22) compared to those without infection with 12.5 days (IQR: 10 - 18; *P* =
234 .006).

235

236 **Active HHV-6 infection and other complications**

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

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

248 Although diarrhoea and mucositis were more frequent in non HHV-6 infected patients
249 than in infected ones (Table 3), active HHV-6 infection was associated with an increased
250 number of combined non-infectious complications (OR 5.05; 95%CI 1.78-14.32; $P < .001$).
251 Moreover, the severity of these complications was higher in this group with more grade
252 3-4 complications (OR 4.62; 95%CI 1.32-16.2; $P = .006$).

253 **DISCUSSION**

254

255 To date, this study is the first large-scaled multicentre prospective non-randomized
256 study including 196 autologous stem cell transplants recipients. The first aim was to
257 determine the incidence of active HHV-6 infection: it was of 11.2% with a cumulative
258 incidence of 19% at 40 days after transplantation. Few studies had already addressed
259 this question in the setting of ASCT: the retrospective works of Imbert-Macille et al.,
260 Inazawa et al. and more recently Colombier et al. reported an incidence of HHV-6
261 infection of 42.5%, 11.4%, and 8.5%, respectively(9, 23, 31). All defined the presence of
262 any level of HHV-6 DNA in blood as active HHV-6 infection. In our work, in order to
263 overcome blips of DNAemia, 2 consecutive blood HHV-6 DNAemias were needed to
264 assess the diagnosis of active HHV-6 infection and to appreciate its kinetics. Up to date,
265 no threshold has been formally recognised as the frontier between latent infection and
266 active infection: in order not to omit low reactivations, we opted for a reference value of
267 blood HHV-6 DNAemias ≥ 450 copies/mL. By using the same criteria as in the studies
268 listed just above, the HHV-6 incidence raised to almost 30% in our study. In accordance
269 with Imbert-Marcille et al. who assessed that active HHV-6 infection frequently occurred
270 early after transplantation with a median of 16 days in the ASCT cohort(9), HHV-6
271 infection occurred with a median time of 13 days in our cohort.

272 HHV-6 and CMV DNAemias are either monitored on whole blood or plasma
273 specimen, depending on the choice of the laboratory. In Europe, and especially in
274 France(4, 22, 23, 29, 32, 33), whole blood is the first used, mainly because this specimen
275 has very limited preparation phases at the preanalytical step (no isolation of leucocytes
276 and no centrifugation of plasma that could lyse cells(1)). This specimen can also be used
277 in case of agranulocytosis, allows the detection of virus replication earlier, and allows

278 the detection of the ci-HHV-6 when present (4, 6). Most studies cited above monitored
279 HHV-6 in whole blood specimen(23, 31, 34). Although detection of viral mRNA could be
280 useful to analyse latent (35), this tool is not currently used in routine and consequently
281 we could not conclude on the presence of latent infection in our patients.

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

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

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

298 HHV-6 infection occurred more frequently in patients with BEAM conditioning
299 regimen. However, BEAM is more used for lymphoma in which immunity was probably
300 lower than plasmocytoma disorder in part because of immunotherapy as rituximab used
301 before. Moreover, due to supply difficulties of Carmustine, some patients (n=16)
302 received Bendamustine, an immunosuppressive agent combining alkylating and

303 antimetabolite properties known to cause T-cell lymphopenia(37). By now, it is too early
304 to assess whether this regimen (Bendamustine-EAM) promoted viral infections but
305 vigilance regarding this question is required in the future.

306 We **also** hypothesized that HHV-6 infections may correlate with other
307 opportunistic challenging viruses such as CMV. **Both CMV and HHV-6 are lymphotropic**
308 **viruses and are reported to be simultaneously or successively detected in allo-SCT**
309 **recipients(12, 22)**. In our cohort, only one patient had concomitant CMV **and HHV-6**
310 infections. This low association is concordant with previous **studies**: Jeulin et al. showed
311 that HHV-6 DNAemia was not significantly associated with CMV infection in a cohort of
312 220 allo-SCT patients including 44 HHV-6 infections(32); Horowitz et al. **also** showed
313 only one patient with concurrent reactivation of CMV out of the **10 ASCT** patients
314 diagnosed with HHV-6 reactivation(38).

315 **The second objective of our study was to analyse hematopoietic reconstitution in**
316 **ASCT patients**. CD34⁺ hematopoietic progenitors can **indeed** carry latent HHV-6 and
317 hematopoietic differentiation can lead to HHV-6 reactivation giving an explanation for
318 myelosuppression(39). In allo-SCT recipients, presence of HHV-6 DNA was significantly
319 associated with delayed platelet and neutrophil engraftment(8, 22). **In our cohort of**
320 **ASCT patients, we observed** a delay in platelets and **ANC** reconstitution with
321 **consequences** on durations of hospitalisations and need of late transfusions in patients
322 with HHV-6 infections, **potentially increasing the costs**. One tricky point is **that this**
323 delayed hematopoietic reconstitution occurred before HHV-6 reactivation. However, it is
324 difficult to precisely date the onset time of HHV-6 infection in clinical practice: as our
325 definition of HHV-6 infection was very stringent and took 7 days, HHV-6 could have
326 clinical consequences even at infra-biological thresholds as seen with CMV(40), or at the
327 moment of the virus reactivation during the week apart between the 2 **measurements**.

328 This is one of the explanations of the occurrence of **delayed** neutropenia recovery prior
329 to the median of onset time of HHV-6 infection: neutropenia recovery is delayed by 2
330 days (10 versus 8 days) during HHV-6 infection while the median of onset time of HHV-6
331 infection is 13 days.

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

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

347
348 **ACKNOWLEDGMENTS SECTION**

349 **Conflict of interest statement:** There are no conflicts of interest to report.

350

351 **Authorship statement:**

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

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

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

358 Emmanuelle Tavernier, Victoria Cacheux, Cécile Moluçon-Chabrot and Karine Augeul-
359 Meunier provided clinical care, recorded clinical data and commented on the
360 manuscript.
361 Vanessa Escuret, Audrey Mirand and Christel Regagnon performed biological analyses.
362 Fabien Tinquaut performed statistical analyses.
363 Gilles Salles and Bruno Pozzetto wrote and revised the manuscript.
364 Jacques-Olivier Bay and Denis Guyotat commented on the manuscript.
365

366 **Funding**

367 **This research has received funding support from the Institut de Cancérologie**
368 **Lucien Neuwirth and the Ligue contre le cancer.**
369

370

371 **Acknowledgments**

372 **The authors are grateful to Prof Henri Agut for helpful discussions.**

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REFERENCES

1. Hill JA, Sedlak RH, Jerome KR. Past, present, and future perspectives on the diagnosis of Roseolovirus infections. *Curr Opin Virol.* 2014;9:84-90.
2. Hill JA, Zerr DM. Roseoloviruses in transplant recipients: clinical consequences and prospects for treatment and prevention trials. *Curr Opin Virol.* 2014;9:53-60.
3. Agut H, Bonnafous P, Gautheret-Dejean A. Human Herpesviruses 6A, 6B, and 7. *Microbiol Spectr.* 2016;4(3).
4. Agut H, Bonnafous P, Gautheret-Dejean A. Laboratory and clinical aspects of human herpesvirus 6 infections. *Clin Microbiol Rev.* 2015;28(2):313-35.
5. Isomura H, Yoshida M, Namba H, Yamada M. Interaction of human herpesvirus 6 with human CD34 positive cells. *J Med Virol.* 2003;70(3):444-50.
6. Pellett PE, Ablashi DV, Ambros PF, Agut H, Caserta MT, Descamps V, et al. Chromosomally integrated human herpesvirus 6: questions and answers. *Rev Med Virol.* 2012;22(3):144-55.
7. Caserta MT, McDermott MP, Dewhurst S, Schnabel K, Carnahan JA, Gilbert L, et al. Human herpesvirus 6 (HHV6) DNA persistence and reactivation in healthy children. *J Pediatr.* 2004;145(4):478-84.
8. de Pagter PJ, Schuurman R, Meijer E, van Baarle D, Sanders EA, Boelens JJ. Human herpesvirus type 6 reactivation after haematopoietic stem cell transplantation. *J Clin Virol.* 2008;43(4):361-6.
9. Imbert-Marcille BM, Tang XW, Lepelletier D, Besse B, Moreau P, Billaudel S, et al. Human herpesvirus 6 infection after autologous or allogeneic stem cell transplantation: a single-center prospective longitudinal study of 92 patients. *Clin Infect Dis.* 2000;31(4):881-6.
10. Fernandez-Ruiz M, Kumar D, Husain S, Lilly L, Renner E, Mazzulli T, et al. Utility of a monitoring strategy for human herpesviruses 6 and 7 viremia after liver transplantation: a randomized clinical trial. *Transplantation.* 2015;99(1):106-13.
11. Knox KK, Carrigan DR. Disseminated active HHV-6 infections in patients with AIDS. *Lancet.* 1994;343(8897):577-8.
12. Agut H. Deciphering the clinical impact of acute human herpesvirus 6 (HHV-6) infections. *J Clin Virol.* 2011;52(3):164-71.
13. Ogata M, Fukuda T, Teshima T. Human herpesvirus-6 encephalitis after allogeneic hematopoietic cell transplantation: what we do and do not know. *Bone Marrow Transplant.* 2015;50(8):1030-6.
14. Hill JA, Myerson D, Sedlak RH, Jerome KR, Zerr DM. Hepatitis due to human herpesvirus 6B after hematopoietic cell transplantation and a review of the literature. *Transpl Infect Dis.* 2014;16(3):477-83.
15. Gotoh M, Yoshizawa S, Katagiri S, Suguro T, Asano M, Kitahara T, et al. Human herpesvirus 6 reactivation on the 30th day after allogeneic hematopoietic stem cell transplantation can predict grade 2-4 acute graft-versus-host disease. *Transpl Infect Dis.* 2014;16(3):440-9.
16. Phan TL, Carlin K, Ljungman P, Politikos I, Boussiotis V, Boeckh M, et al. Human Herpesvirus-6B Reactivation Is a Risk Factor for Grades II to IV Acute Graft-versus-Host Disease after Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. *Biol Blood Marrow Transplant.* 2018;24(11):2324-36.
17. Zerr DM, Boeckh M, Delaney C, Martin PJ, Xie H, Adler AL, et al. HHV-6 reactivation and associated sequelae after hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* 2012;18(11):1700-8.

- 422 18. Dulery R, Salleron J, Dewilde A, Rossignol J, Boyle EM, Gay J, et al. Early human
423 herpesvirus type 6 reactivation after allogeneic stem cell transplantation: a large-scale
424 clinical study. *Biol Blood Marrow Transplant.* 2012;18(7):1080-9.
- 425 19. Shimazu Y, Kondo T, Ishikawa T, Yamashita K, Takaori-Kondo A. Human
426 herpesvirus-6 encephalitis during hematopoietic stem cell transplantation leads to poor
427 prognosis. *Transpl Infect Dis.* 2013;15(2):195-201.
- 428 20. Attal M, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, et al. A
429 prospective, randomized trial of autologous bone marrow transplantation and
430 chemotherapy in multiple myeloma. *Intergroupe Francais du Myelome. N Engl J Med.*
431 1996;335(2):91-7.
- 432 21. Peinemann F, Enk H, Smith LA. Autologous hematopoietic stem cell
433 transplantation following high-dose chemotherapy for nonrhabdomyosarcoma soft
434 tissue sarcomas. *Cochrane Database Syst Rev.* 2017;4:CD008216.
- 435 22. Quintela A, Escuret V, Roux S, Bonnafous P, Gilis L, Barraco F, et al. HHV-6
436 infection after allogeneic hematopoietic stem cell transplantation: From chromosomal
437 integration to viral co-infections and T-cell reconstitution patterns. *J Infect.*
438 2016;72(2):214-22.
- 439 23. Colombier MA, Amorim S, Salmona M, Thieblemont C, Legoff J, Lafaurie M. HHV-6
440 reactivation as a cause of fever in autologous hematopoietic stem cell transplant
441 recipients. *J Infect.* 2017;75(2):155-9.
- 442 24. Schlaweck S, Bragelmann J, Brossart P, Mayer K. Exanthem subitum (human
443 herpesvirus-6 reactivation) after autologous stem cell transplantation. *Transpl Infect*
444 *Dis.* 2016;18(2):255-6.
- 445 25. Chapenko S, Trociukas I, Donina S, Chistyakov M, Sultanova A, Gravelina S, et al.
446 Relationship between beta-herpesviruses reactivation and development of
447 complications after autologous peripheral blood stem cell transplantation. *J Med Virol.*
448 2012;84(12):1953-60.
- 449 26. Styczynski J, Czyzewski K, Wysocki M, Gryniewicz-Kwiatkowska O, Kolodziejczyk-
450 Gietka A, Salamonowicz M, et al. Increased risk of infections and infection-related
451 mortality in children undergoing haematopoietic stem cell transplantation compared to
452 conventional anticancer therapy: a multicentre nationwide study. *Clin Microbiol Infect.*
453 2016;22(2):179 e1- e10.
- 454 27. Fule Robles JD, Cheuk DK, Ha SY, Chiang AK, Chan GC. Human herpesvirus types 6
455 and 7 infection in pediatric hematopoietic stem cell transplant recipients. *Ann*
456 *Transplant.* 2014;19:269-76.
- 457 28. Dueck AC, Mendoza TR, Mitchell SA, Reeve BB, Castro KM, Rogak LJ, et al. Validity
458 and Reliability of the US National Cancer Institute's Patient-Reported Outcomes Version
459 of the Common Terminology Criteria for Adverse Events (PRO-CTCAE). *JAMA Oncol.*
460 2015;1(8):1051-9.
- 461 29. Deback C, Agbalika F, Scieux C, Marcelin AG, Gautheret-Dejean A, Cherot J, et al.
462 Detection of human herpesviruses HHV-6, HHV-7 and HHV-8 in whole blood by real-
463 time PCR using the new CMV, HHV-6, 7, 8 R-gene kit. *J Virol Methods.* 2008;149(2):285-
464 91.
- 465 30. Ogata M, Kikuchi H, Satou T, Kawano R, Ikewaki J, Kohno K, et al. Human
466 herpesvirus 6 DNA in plasma after allogeneic stem cell transplantation: incidence and
467 clinical significance. *J Infect Dis.* 2006;193(1):68-79.
- 468 31. Inazawa N, Hori T, Nojima M, Saito M, Igarashi K, Yamamoto M, et al. Virus
469 reactivations after autologous hematopoietic stem cell transplantation detected by
470 multiplex PCR assay. *J Med Virol.* 2017;89(2):358-62.

471 32. Jeulin H, Agrinier N, Guery M, Salmon A, Clement L, Bordigoni P, et al. Human
472 herpesvirus 6 infection after allogeneic stem cell transplantation: incidence, outcome,
473 and factors associated with HHV-6 reactivation. *Transplantation*. 2013;95(10):1292-8.

474 33. Pillet S, Roblin X, Cornillon J, Mariat C, Pozzetto B. Quantification of
475 cytomegalovirus viral load. *Expert Rev Anti Infect Ther*. 2014;12(2):193-210.

476 34. Roux J, Battistella M, Fornecker L, Legoff J, Deau B, Houhou N, et al. Human
477 Herpesvirus-6 cytopathic inclusions: an exceptional and recognizable finding on skin
478 biopsy during HHV6 reactivation after autologous stem-cell transplantation. *Am J*
479 *Dermatopathol*. 2012;34(6):e73-6.

480 35. Hill JA, Ikoma M, Zerr DM, Basom RS, Peddu V, Huang ML, et al. RNA Sequencing
481 of the In Vivo Human Herpesvirus 6B Transcriptome To Identify Targets for Clinical
482 Assays Distinguishing between Latent and Active Infections. *J Virol*. 2019;93(3).

483 36. Ljungman P, Wang FZ, Clark DA, Emery VC, Remberger M, Ringden O, et al. High
484 levels of human herpesvirus 6 DNA in peripheral blood leucocytes are correlated to
485 platelet engraftment and disease in allogeneic stem cell transplant patients. *Br J*
486 *Haematol*. 2000;111(3):774-81.

487 37. Gafter-Gvili A, Polliack A. Bendamustine associated immune suppression and
488 infections during therapy of hematological malignancies. *Leuk Lymphoma*.
489 2016;57(3):512-9.

490 38. Horowitz N, Oren I, Lavi N, Zuckerman T, Benyamini N, Kra-Oz Z, et al. New rising
491 infection: human herpesvirus 6 is frequent in myeloma patients undergoing autologous
492 stem cell transplantation after induction therapy with bortezomib. *Bone Marrow Res*.
493 2012;2012:409765.

494 39. Andre-Garnier E, Milpied N, Boutolleau D, Saiagh S, Billaudel S, Imbert-Marcille
495 BM. Reactivation of human herpesvirus 6 during ex vivo expansion of circulating CD34+
496 haematopoietic stem cells. *J Gen Virol*. 2004;85(Pt 11):3333-6.

497 40. Razonable RR, Hayden RT. Clinical utility of viral load in management of
498 cytomegalovirus infection after solid organ transplantation. *Clin Microbiol Rev*.
499 2013;26(4):703-27.

500

FIGURE LEGEND

Figure 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

Figure 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.

Figure 1:

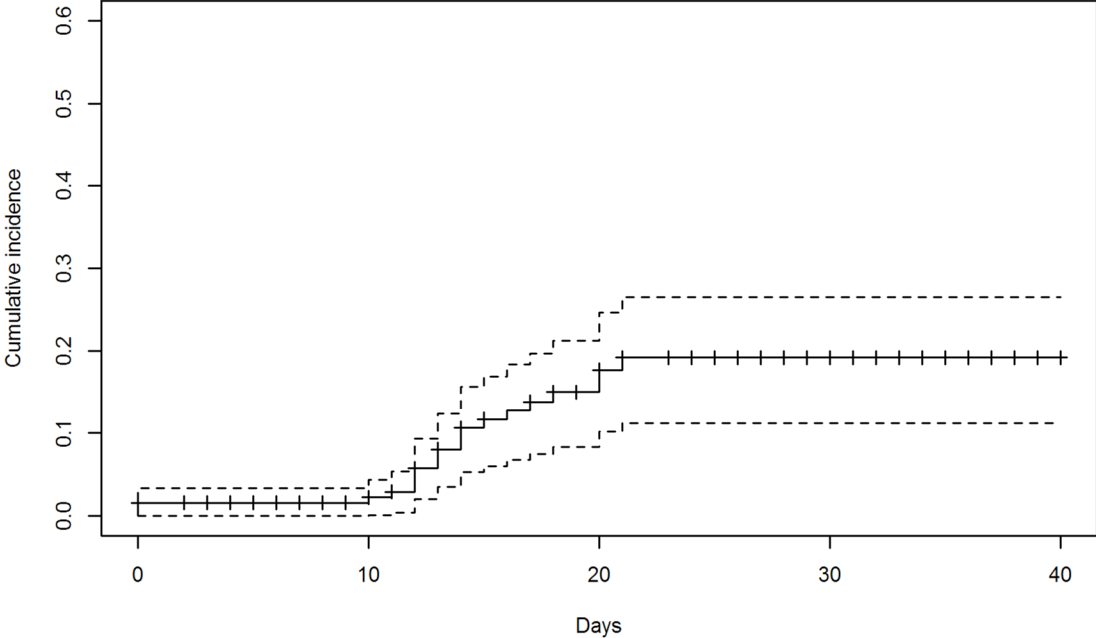
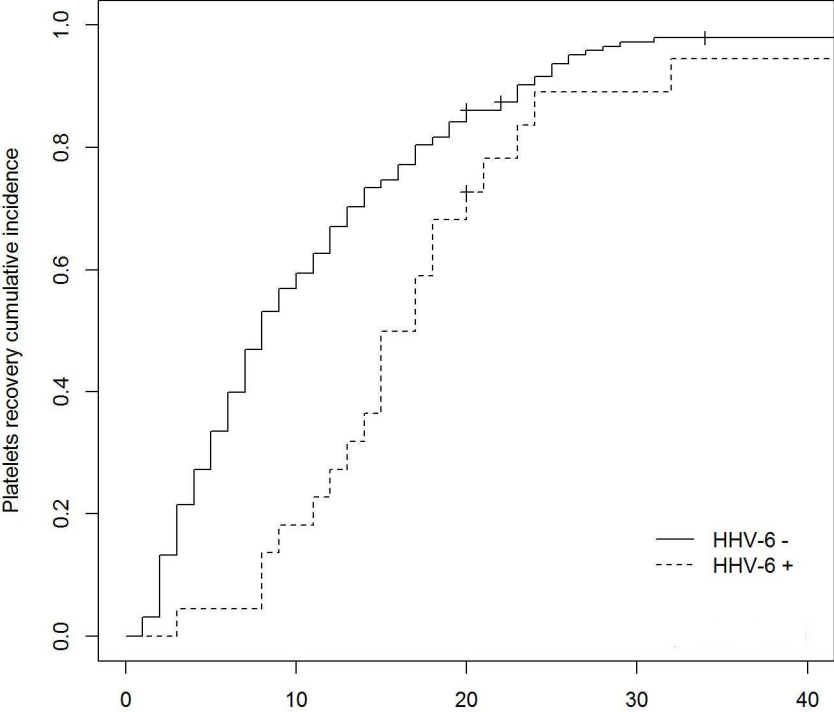
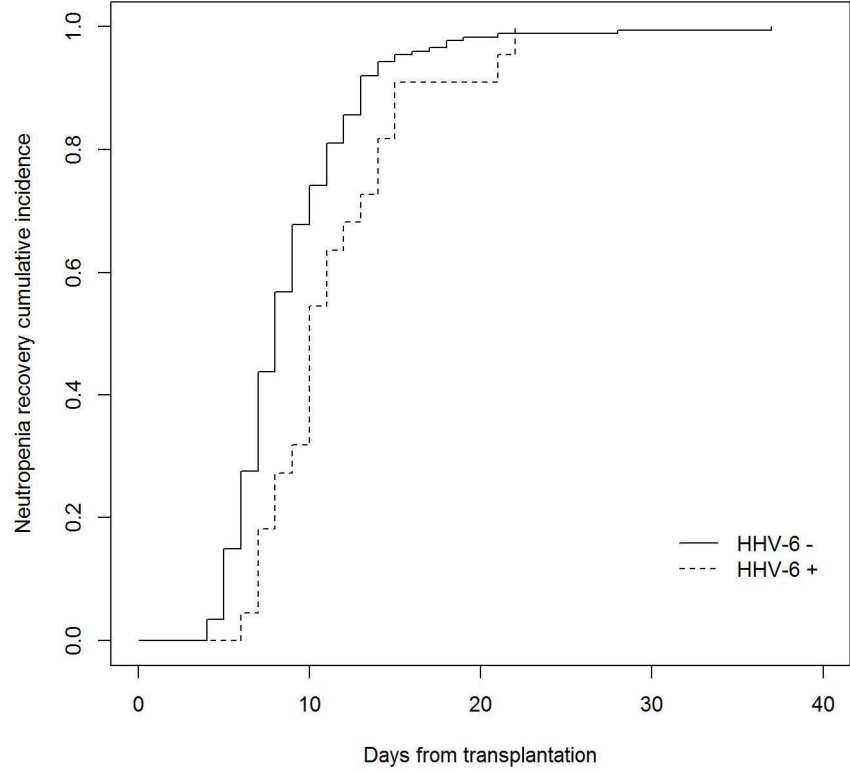


Figure 2:

A



B



TABLES

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

	Non-infected patients (n=174) n (%)	HHV-6-infected patients (n=22) n (%)	TOTAL (n=196) n (%)	P-value
Patients' Baseline Characteristics,				
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.

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-infectious patients n (%)	HHV-6-infected patients n (%)	Total n (%)
Non infectious complications			
<i>Oral mucositis</i>	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%)
<i>Diarrhea</i>	127 (73%)	9 (40.9%)	136 (69.4%)
<i>Liver enzyme elevation</i>	111 (63.8%)	16 (72.7%)	127 (64.8%)
<i>Skin rash</i>	58 (33.3%)	11 (50%)	69 (35.2%)
<i>Acute kidney injury</i>	23 (13.2%)	4 (18.2%)	27 (13.8%)
Infectious complications			
<i>Febrile neutropenia</i>	109 (62.6%)	15 (68.2%)	124 (63.3%)
<i>Clinically/microbiologically sites involved</i>			
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%)