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Impact of stem cell graft on early viral infections and immune reconstitution after allogeneic transplantation in adults

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ABSTRACT

Background: Viral infections are well-known complications after allogeneic stem cell transplant (allo-SCT).
Objectives: We compared prospectively incidences of DNAemia and active infections (AI) for five opportunistic viruses (Human Herpesvirus 6 (HHV-6), Epstein-Barr virus (EBV), BK polyomavirus (BKPyV), Cytomegalovirus (CMV) and Adenovirus (ADV)) and kinetics of immune reconstitution (IR) in adults receiving either double umbilical cord blood (dUCB group) or unrelated peripheral blood stem cell (uPBSC group) allo-SCT after a reduced-intensity conditioning (RIC) regimen.
Study design: Whole blood samples were collected at transplant, every 15 days during the first 3 months and at 4, 5 and 6 months post-transplant.
Results: Sixty-five patients were enrolled (uPBSC n = 34; dUCB n = 31). Incidences of HHV-6 and BKPyV DNAemia were significantly higher for dUCB (97% vs 23.5% and 58% vs 32%, respectively) while EBV DNAemia was more frequently detected in uPBSC (71% vs 26%). The incidence of CMV DNAemia was similar between both groups. ADV AI developed only in dUCB. HHV-6 AI were also higher in dUCB (84% vs 21%). In multivariate analysis, dUCB graft was the only independent factor associated with HHV-6 DNAemia (OR: 19.0; 95%CI: 5.2–69.1; $p < 0.0001$) while EBV DNAemia were significantly associated with uPBSC (OR: 29.9; 95%CI: 5.68–158; $p < 0.0001$). dUCB graft was also the only factor associated with HHV-6 AI. Finally, higher counts and faster recoveries of B lymphocytes ($p < 0.0001$) and monocytes ($p = 0.0007$) were observed in the dUCB group.
Conclusion: We demonstrate a strong correlation between sources of graft and patterns of viral DNAemia and AI and IR after RIC allo-SCT.

1. Introduction

Over recent decades, umbilical cord blood (UCB) has become a well-established alternative source of hematopoietic stem cells (HSC) in patients without available bone marrow or peripheral blood stem cell (PBSC) grafts [1]. Because of the lower quantity of HSC and the naïve status of infused T cells, UCB allogeneic stem cell transplantation (allo-SCT) can be associated with delayed engraftment, poor immune reconstitution (IR), and higher incidence of opportunistic infections compared to transplants using conventional sources of HSC [2,3].

Five main opportunistic viruses cause complications after allo-SCT: human Cytomegalovirus (CMV), Epstein-Barr Virus (EBV) Human

Abbreviations: ADV, adenovirus; Allo-SCT, allogeneic stem cell transplant; ATG, antithymocyte globulins; BKPyV, BK polyomavirus; CI, confidence interval; CMV, cytomegalovirus; DNA, deoxyribonucleic acid; DNAemia, detection of viral DNA in blood; dUCB, double umbilical cord blood; EBV, Epstein Barr virus; GVHD, graft-versus-host disease; HHV-6, human herpesvirus 6; HSC, hematopoietic stem cells; IR, immune reconstitution; OR, odds ratio; PBSC, peripheral blood stem cell; PCR, polymerase chain reaction; PTLD, post-transplant lymphoproliferative disorder; RIC, reduced-intensity conditioning; TRM, transplant-related mortality; uPBSC, unrelated peripheral blood stem cell; VL, viral load; WB, whole blood

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Herpesvirus 6 (HHV-6); BK polyomavirus (BKPyV) and adenovirus (ADV). Viral infections are the consequence of delayed IR post-transplantation and remain a matter of concern as they are associated with high morbidity and mortality rates [4,5]. HHV-6 may cause encephalitis [6] while CMV is associated mainly with colitis, retinitis, meningo-encephalitis and pneumonia [7], EBV with post-transplant lymphoproliferative disorders (EBV-PTLD) [8], BKPyV with hemorrhagic cystitis [9] and ADV with fever, diarrhea, hepatitis, pneumonia and cystitis [10].

Currently, only limited data are available concerning the role of HSC source on the occurrence of viral infections after allo-SCT. In a retrospective study, we showed that HHV-6 infections were more frequently observed after double UCB (dUCB) than after unrelated PBSC (uPBSC) allo-SCT in adults, while no correlation could be established for CMV or EBV [11]. Since then, the specific relationship between HHV-6 and dUCB transplant has been widely reported [12–15]. Also, in our study, higher counts of B lymphocytes were found after dUCB grafts while CD8+ T-cells counts were higher using PBSC as HSC source, up to 9 and 6 months post-transplant, respectively [11].

Here, to confirm our data, we conducted a prospective study comparing the pattern of viral infections mentioned above, as well as the IR, of patients receiving either a dUCB or uPBSC allo-SCT after a reduced intensity conditioning (RIC) regimen, which is currently the main type of regimen used in adults [16].

2. Patients and methods

2.1. Inclusion criteria and study design

Between December 2009 and August 2012, all consecutive adults (≥18 years old) programmed to receive either a dUCB or an uPBSC allo-SCT after a RIC regimen in Nantes University Hospital were prospectively proposed to participate to the study. All types of disease, disease status or RIC regimens [17] were permitted. The main objective was to compare between both groups the incidences of 5 viral infections (HHV-6, CMV, EBV, ADV and BKPyV), up to 6 months post-transplant, and to evaluate their impact on clinical symptoms and other outcomes (neutrophil and platelet recoveries, relapse, survival, acute graft-versus-host disease (aGVHD). Chronic GVHD, overall and disease free survivals were not considered in this study. The secondary objective was to compare IR up to the end of the follow-up. Whole blood (WB) samples for viral and immunological analyses were collected at transplant, every 15 days during the first 3 months and at 4, 5 and 6 months post-transplant (10 samples expected/patient). All patients provided informed consent, and the study was approved by the review board of Nantes University Hospital.

2.2. Definitions

2.2.1. Hematopoietic recovery, engraftment and GVHD

Definitionof neutrophil and platelet recoveries is given in **Supplemental file**. Acute GVHD were diagnosed and graded according to standard criteria [18].

2.2.2. Viral DNAemia, active infection and disease, and antiviral prophylaxis and treatment

Viral DNAemia was defined as any positive PCR in whole blood, whatever the viral load (VL) or number of positive samples. Among, the patients with positive DNAemia, active infection (AI) was defined by having at least two consecutive PCR with a viral load (VL) > 3 $\log_{10}$, expressed as number of viral DNA copies ($\log_{10}$cop) per mL of WB, per 10^6 cells or per 10^5 cells according to the virus (see below) [11].

Viral disease was defined as any detection of virus by PCR or immunohistochemistry in normally virus-free body fluids or organ tissues, together with symptoms and/or signs from the affected organ [4,19,20]. EBV-PTLD was defined as biopsy proven lymphoma or EBV reactivation along with computerized tomography nodal or soft-tissue abnormalities consistent with lymphoproliferative disorder [5].

During the study, all patients received valaciclovir (1 g per day) as herpes simplex virus and varicella-zona virus prophylaxis. Antiviral strategy was applied according the European Conference on Infections in Leukemia guidelines [4,5,19].

2.3. Viral and immune monitoring after transplant

Virus DNA quantifications were performed using in-house real-time procedures adapted from previously described protocols [21–24]. VL were expressed in $\log_{10}$cop per ml of WB for HHV-6, BKPyV and ADV, or per 10^6 and 10^5 cells, for CMV and EBV, respectively.

Circulating lymphocyte numbers were measured by flow cytometry on a BD FacsCanto II analyzer and determined with BD FACSCanto Clinical Software (BD Biosciences) (**Supplemental file**).

2.4. Statistical analysis

Patients' characteristics, transplant-related events and association of each viral DNAemia, AI or viral disease if required were evaluated with relevant variables by univariate analysis, using either the chi-squared test or the two-tailed Fisher's exact test for proportions, and the Wilcoxon test for quantitative data. Risk factors for developing DNAemia associated with a p value < 0.20 on univariate analysis were included in the final logistic regression model for multivariate analysis. Only engrafted patients were considered for IR, which was analysed using mixed linear models. A p value < 0.05 was considered as statistically significant. All statistical analyses were performed using SAS 9.3 (SAS Institute Inc., NC, USA).

3. Results

3.1. Patients

Sixty-five consecutive patients were enrolled in the study (uPBSC n = 34; dUCB n = 31). Regarding the characteristics of patients (Table 1), both groups differed by higher use of rabbit antithymocyte globulines (ATG) in uPBSC, a lower number of HSC infused in dUCB, the types of RIC regimen and GVHD prophylaxis. Details of RIC regimens [25–28] and GVHD prophylaxis are given in Table 1. Comparison of outcomes is given in Table 2.

3.2. Positive DNAemia, AI and disease: risk factors and influence on outcome

During the 6-month follow-up, a total number of 608 samples were analyzed and collection of samples has been completed for a large majority of patients (median: 10/patient). Among the whole cohort, 38 (59%), 32 (49%), 29 (45%), 24 (37%), and 4 (6%) patients experienced positive DNAemia for HHV-6, EBV, BKPyV, CMV and ADV, respectively. Only 6 (9%) patients remained free of positive DNAemia, including 5 uPBSC cases, whereas positive DNAemia for 2, 3 and 4 viruses were observed for 30, 10 and 3 patients respectively. No associations between different pairs of viral infections, or between viral infections and aGVHD or death were established.

Data collected for viral monitoring are summarized in Table 3. HHV-6 (Fig. 1**A**), BKPyV (Fig. 1**B**) and ADV DNAemia were associated with dUCB while EBV DNAemia (Fig. 1**C**) were associated with uPBSC. CMV DNAemia (Fig. 1**D**) had the same incidences in both groups. More details are provided in **Supplemental file** regarding incidence of viral AI and diseases and comparison between both groups.

3.3. IR and comparison between both groups

IR was considered only in engrafted patients (dUCB = 27; uPBSC