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Cytomegalovirus Infection in Patients Who Underwent Allogeneic Hematopoietic Stem Cell Transplantation in Portugal: A Five-Year Retrospective Review

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A B S T R A C T

Cytomegalovirus (CMV) infection is 1 of the leading causes of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (aHSCT), mainly within the first 100 days after transplantation. We aimed to characterize CMV infection in a cohort of 305 patients with different malignancies undergoing aHSCT at the Portuguese Institute of Oncology of Porto between January 2008 and December 2012. In total, 184 patients (60.3%) developed CMV infection, mainly viral reactivations rather than primary infections (96.2% versus 3.8%, respectively). The majority of patients (166 of 184) developed CMV infection ≤100 days after transplantation, with median time to infection of 29 days (range, 0 to 1285) and median duration of infection of 10 days (range, 2 to 372). Multivariate analysis revealed that CMV infection was increased in donor (D)-/recipient (R)+ and D+/R+ (odds ratio [OR], 10.5; 95% confidence interval [CI], 4.35 to 25.4; $P < .001$) and in patients with mismatched or unrelated donors (OR, 2.54; 95% CI, 1.34 to 4.80; $P = .004$). Cox regression model showed that the risk of death was significantly increased in patients >38 years old (OR, 1.89; 95% CI, 1.14 to 3.12; $P = .0137$), who underwent transplantation with peripheral blood (OR, 3.02; 95% CI, 1.33 to 6.86; $P = .008$), with mismatched or unrelated donor (OR, 2.16; 95% CI, 1.48 to 3.13; $P < .001$), and who developed CMV infection (OR, 1.76; 95% CI, 1.07 to 2.90; $P = .025$). Moreover, patients who developed CMV infection had a significantly reduced median post-transplantation survival (16 versus 36 months; $P = .002$).

INTRODUCTION

Viral infections are currently 1 of the major causes of morbidity and mortality in patients undergoing hematopoietic stem cell transplantation (HSCT), and therefore, viral monitoring has a great impact on HSCT patients [1-7]. Cytomegalovirus (CMV) infection is currently recognized as a leading cause of morbidity and mortality after allogeneic HSCT (aHSCT), as it is frequently associated with multiorgan disease, including pneumonia, hepatitis, gastroenteritis, retinitis, and encephalitis [8-12]. Nevertheless, not all individuals are at the same risk of CMV complication, as the risk of infection/disease varies with age, source of transplantation, underlying disease, donor (D)/recipient (R) CMV serological status, and occurrence of graft-versus-host disease (GVDH) [13,14].

Typically, CMV infection appears within the first 100 days after HSCT and affects mainly the lungs and the gastrointestinal tract [4,10,12,15,16]. However, with the introduction of antiviral drugs, an increasing number of infections occur in a late period exceeding 100 days after HSCT [7,8]. Hence, the introduction of reliable and sensitive laboratory tests for early detection of CMV infection that allow the start of

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efficient preemptive therapy has contributed to a reduction of post-transplantation morbidity and mortality [11,17-20].

Despite advances in diagnosis and prevention, CMV infection it is still considered a major problem for aHSCT patients [2,10,11,21]. Hence, the aim of the present study was to retrospectively review and characterize CMV infection among aHSCT recipients by summarizing the data from consecutive patients undergoing aHSCT at the Portuguese Institute of Oncology of Porto (IPO Porto) during the period of 2008 to 2012.

MATERIAL AND METHODS

Type of Study and Population

We performed a retrospective study in a cohort of 305 patients with different malignancies undergoing aHSCT at the bone marrow transplantation service from the Portuguese Institute of Oncology of Porto between January 2008 and December 2012. This study was approved by the institution ethical committee (ref CES 462/013) and clinical data were collected retrospectively from the clinical records of patients.

Transplantation Procedures

Transplantation was performed according to institutional protocols based on international standards for aHSCT. Briefly, the myeloablative regimen was based on busulfan and cyclophosphamide, whereas the reduced-intensity conditioning (RIC) was based on a reduced busulfan dose plus fludarabine. Patients with mismatched or unrelated donors received antithymocyte globulin as part of the conditioning regimen. GVHD prophylaxis in patients on a myeloablative regimen consisted of a combination of a calcineurin inhibitor, either cyclosporine (CsA) or tacrolimus, and short-term methotrexate. Patients using RIC received CsA or tacrolimus plus mycophenolate mofetil. GVHD prophylaxis was maintained for 6 months with a progressive reduction of immunosuppression: CsA and tacrolimus were reduced by one third each month after the third month, and mycophenolate mofetil was reduced by one half at day +29, by one quarter at day +57, and suspended at day +85.

CMV Monitoring after aHSCT

All patients were followed for CMV infection according to the institutional protocol for aHSCT patients. Prospective post-transplantation CMV monitoring was performed in peripheral blood samples starting at day of transplantation with biweekly analysis up to 100 days or longer if complications were present, weekly up to 180 days, bimonthly up to 1 year, and after the first year on routine evaluations.

CMV infection monitoring was performed on whole blood samples by pp65 antigenemia assay, with the C10/C11 monoclonal antibody cocktail (IQ Products, Groningen, Netherlands) to detect the CMV lower matrix phosphoprotein (pp65) or, if WBC count was <1000/mL by quantitative real-time PCR using the Q-CMV Real Time Complete kit (ELITech Group, Puteaux, France).

Management of Post-aHSCT CMV Infection

All patients were followed using a preemptive strategy for CMV disease prevention. Institutional cut-off for initiation of preemptive antiviral therapy during the first 100 days after aHSCT was based on pp65 antigenemia results. Preemptive anti-CMV therapy was initiated after the detection of ≥2 antigen-positive cells per 5.0×10^4 leukocytes or >1000 copies/mL with oral valganciclovir (VGCV) at a dose of 900 mg twice a day for 14 days as induction therapy. In the context of gastrointestinal intolerance, oral VGCV was substituted with intravenous ganciclovir (GCV) at a dose of 5 mg/kg twice daily for 14 days. All patients received one-half dose of antiviral (VGCV 900 mg or GCV 5 mg/kg once a day) as a maintenance therapy until negative CMV detection or day 100. Patients with severe VGCV- or GCV-associated cytopenia were treated with intravenous foscarnet at a dose of 60 mg/kg/8 hours for 14 days. Antiviral doses were closely monitored according to patients' renal function and results from pp65 antigenemia or CMV viral load.

Variable Definitions

Hematological diseases were classified into the following categories: (1) acute leukemia (acute myeloid leukemia, acute lymphoid leukemia), (2) chronic myeloproliferative disorders (chronic myeloid leukemia, polycythemia vera, myelofibrosis), (3) chronic lymphoproliferative disorders (B cell lymphoma, T cell lymphoma, multiple myeloma, chronic lymphoid leukemia), (4) myelodysplastic syndrome (chronic myelomonocytic leukemia), (5) aplastic anemia (Fanconi anemia, dyskeratosis congenita, Diamond-Blakfan syndrome), and (6) others (metabolic disease, hemoglobinopathies). The status of disease at the time of aHSCT was classified as follows: (1) first complete remission or inactive disease after the first-line treatment, (2) second or following complete remission after equal or greater than second-line treatment, and (3) relapse or active disease.

Table 1
Baseline Characteristics of the Study Population

Variable	n (%)
Gender	
Female	119 (39.0)
Male	186 (61.0)
Age	
≤20 yr old	82 (26.9)
21-40 yr old	85 (27.9)
>40 yr old	138 (45.2)
Underlying disease	
Acute leukemia	161 (52.8)
Chronic myeloproliferative diseases	26 (8.5)
Chronic lymphoproliferative diseases	56 (18.4)
Myelodysplastic/myeloproliferative diseases	28 (8.9)
Aplastic anemia	24 (7.9)
Others	10 (3.6)
Stem cell source	
CB	21 (6.9)
BM	27 (8.9)
PBSC	257 (84.2)
Phase at transplantation (n = 284)	
First RC	199 (70.1)
RC ≥ second	49 (17.2)
Active disease	36 (12.7)
Conditioning regimen	
RIC	120 (39.3)
Myeloablative	185 (60.7)
Donor	
Related	185 (60.7)
Mismatched or unrelated	120 (39.3)
Recipient CMV status	
Seronegative	42 (13.8)
Seropositive	263 (86.2)
Donor CMV status (n = 303)	
Seronegative	81 (26.7)
Seropositive	222 (73.3)
CMV donor/recipient status (n = 303)	
D-/R-	21 (6.9)
D+/R-	22 (7.3)
D-/R+	60 (19.8)
D+/R+	200 (66.0)

CB indicates cord blood; BM, bone marrow; RC, complete remission.

The primary endpoint of this study was to characterize CMV infection after aHSCT. *CMV infection* was defined as a positive result in either pp65 antigenemia (≥1 antigen-positive cell per 5.0×10^4 leukocytes) or real-time PCR (viral load in whole blood sample ≥100 copies/mL). *Time to infection* (TTI) was defined as the difference between the day of aHSCT and the day of first CMV positive result. *Duration of infection* (DOI) was defined as the difference between the day of first positive CMV result and the day of last positive CMV result (that is, the positive result preceding 4 consecutive negative results during biweekly monitoring). *Early CMV infection* was defined as occurring before 100 days after aHSCT, whereas *late CMV infection* was defined as occurring after 100 days from aHSCT.

The outcomes analyzed were engraftment, disease relapse, and mortality. *Acute GVHD* (aGVHD) was defined as occurring during the 100 days after transplantation, and *chronic GVHD* (cGVHD) was defined as occurring after 101 days from transplantation. *Successful engraftment* of transplantation was measured by neutrophil and platelet recoveries, here defined as the first of 3 consecutive days during which the neutrophil and platelet count in blood were over $.5 \times 10^9$/L and 20×10^9/L, respectively, without transfusion support. *Disease relapse* was defined clinically. *Post-transplantation survival* (PTS) was defined as the time between the day of HSCT and the day of the last visit to hospital or the day of death.

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics for Macintosh, Version 20.0 (IBM Corp., Armonk, NY). Chi-square or Fisher exact test were used to compare the categorical variables with a 5% significance level. Univariate analysis and multivariate logistic regression models were used to

estimate odds ratio (OR) and the corresponding 95% confidence intervals (CIs) as a measure of association between the categorical variables, GVHD development, and CMV infection. Multivariate analyses were performed by adjusting for the following covariates: gender, median age at aHSCT, stem cell source, phase at transplantation, conditioning regimen, donor/recipient CMV serological status, HLA mismatch, and, when applicable, GVHD development and CMV infection. Continuous variables were tested for normality with Kolmogorov-Smirnov Test and nonparametric tests (Mann-Whitney U-test) were used for non-normally distributed variables (TTI and DOI). Cox proportional hazard models (univariate and multivariate) were used to assess the risk factors associated with TTI, DOI, disease relapse, and death by estimating hazard ratios and the corresponding 95% CIs. The Kaplan-Meier method with log-rank test was used to calculate the association between CMV infection and PTS.

RESULTS

Characteristics of the Study Population

Clinical variables collected from both patients and donors are summarized in Table 1. In our case series, the median age at aHSCT was 38 years (range, 0 to 66) with 82 patients <18 years of age, 85 patients between 21 and 40 years of age, and 138 patients >40 years of age. The majority of patients underwent aHSCT for acute leukemia (n = 161, 52.8%) followed by chronic lymphoid disease (n = 56, 18.4%). Peripheral blood was the first choice of source of stem cells in 257 cases (84.3%) and related donors were used in 190 cases (62.3%). One hundred and eighty-five (60.7%) underwent myeloablative conditioning regimen before transplantation and 199 were in first complete remission (70.1%). CMV serological status showed that the majority of both patients and donors were seropositive for CMV (n = 200, 67.1%).

GVHD

In our study, a total of 226 patients developed GVHD (75.1%), with 160 (70.8%) aGVHD and 66 (29.2%) cGVHD. We observed that of the 160 patients with aGVHD, 97 (60.6%) developed aGVDH ≥ grade 2 and 102 (63.8%) progressed to cGVHD.

The characterization of GVHD according to clinical variables is shown in Table 2. Univariate analysis revealed that GVHD was more frequent in patients receiving peripheral blood as the source of stem cells ($P = .007$) under myeloablative conditioning ($P = .009$) and in CMV D+/R+ ($P = .044$). The multivariate logistic regression analysis confirmed that patients receiving stem cells from peripheral blood (OR, 2.84; 95% CI, 1.31 to 6.17; $P = .008$) with myeloablative conditioning (OR, 2.04; 95% CI, 1.06 to 3.94; $P = .033$) were associated with increased risk of developing GVHD, and patients in second complete remission or active disease had almost 50% reduction in the probability of developing GVHD (OR, .53; 95% CI, .28 to .99; $P = .048$). Moreover, the analysis confirmed the tendency for increased risk of GVHD development in patients >38 years of age (OR, 1.94; 95% CI, .97 to 3.87; $P = .062$).

CMV Infection

Our case series revealed that 184 patients (60.3%) developed CMV infection, consisting in great majority of viral reactivations rather than primary infections (96.2% versus 3.8%, respectively). Table 3 details the statistical analysis of the association of CMV infection with clinical variables. The statistical analysis revealed no significant association of CMV infection development with gender, age, underlying disease, stem cell source, phase at transplantation, conditioning regimen, donor CMV seropositivity, and GVHD development. Nevertheless, the risk of CMV infection was significantly increased in CMV seropositive recipients ($P < .001$; OR, 10.3; 95% CI, 4.39 to 24.1). When considering the D/R CMV

Table 2
Characterization of GVHD Occurring among aHSCT Recipients

Risk Factor	GVHD n (%)	*P* Value*	OR* (95% CI)	*P* Value†	OR† (95% CI)
Gender					
Female (n = 117)	86 (73.5)	.614	1.15 (.67-1.95)	.874	1.05 (.58-1.88)
Male (n = 184)	140 (76.1)				
Age					
≤38 (n = 152)	107 (70.4)	.058	1.67 (.98-2.84)	.062	1.94 (.97-3.87)
>38 (n = 149)	119 (79.9)				
Stem cell source					
CB or BM (n = 47)	28 (59.6)	**.007**	**2.40 (1.25-4.61)**	**.008**	**2.84 (1.31-6.17)**
PBSC (n = 254)	198 (78.0)				
Phase at transplantation					
RC1 (n = 199)	155 (77.9)	.055	.58 (.33-1.01)	**.048**	**.53 (.28-.99)**
RC ≥ second or active (n = 85)	57 (67.1)				
Conditioning regimen					
RIC (n = 118)	79 (66.9)	**.009**	**2.02 (1.19-3.42)**	**.033**	**2.04 (1.06-3.94)**
Myeloablative (n = 183)	147 (80.3)				
Donor/HLA status					
Related (n = 183)	137 (74.9)	.913	1.03 (.60-1.76)	.274	1.46 (.74-2.89)
Mismatched or unrelated (n = 118)	89 (75.4)				
Recipient CMV status					
Seronegative (n = 42)	30 (71.4)	.555	1.24 (.60-2.58)		
Seropositive (n = 259)	196 (75.7)				
Donor CMV status					
Seronegative (n = 81)	56 (69.1)	.160	1.50 (.85-2.65)		
Seropositive (n = 218)	168 (77.1)				
Donor/recipient CMV status					
D-/R- (n = 21)	17 (81.0)	**.044**‡	**1.73 (1.01-2.96)**	.164	1.55 (.84-2.88)
D+/R- (n = 22)	14 (63.6)				
D-/R+ (n = 60)	39 (65.0)				
D+/R+ (n = 196)	154 (78.6)				

* Univariate analysis.
† Multivariate logistic regression analysis.
‡ The analysis was performed considering D-/R-, D+/R-, and D-/R+ versus D+/R+.

Table 3
Analysis of CMV Infection among aHSCT Recipients

Risk Factor	CMV Infection n (%)	*P* Value*	OR* (95% CI)	*P* Value†	OR† (95% CI)
Gender					
Female (n = 119)	72 (60.5)	.960	.99 (.62-1.58)	.791	1.08 (.63-1.84)
Male (n = 186)	112 (60.2)				
Age					
≤38 (n = 153)	88 (57.5)	.314	1.26 (.80-2.00)	.338	1.34 (.73-2.48)
>38 (n = 152)	119 (63.2)				
Stem cell source					
CB or BM (n = 48)	30 (62.5)	.738	.90 (.48-1.69)	.300	.65 (.28-1.47)
PBSC (n = 257)	154 (59.9)				
Phase at transplantation					
RC1 (n = 199)	115 (57.8)	.367	1.27 (.75-2.15)	.243	1.44 (.78-2.68)
RC ≥ second or active (n = 85)	54 (53.5)				
Conditioning regimen					
RIC (n = 120)	74 (61.7)	.700	.91 (.57-1.46)	.368	.76 (.42-1.38)
Myeloablative (n = 185)	120 (59.5)				
Donor/HLA status					
Related (n = 185)	104 (56.2)	.068	1.56 (.97-2.51)	**.004**	**2.54 (1.34-4.80)**
Mismatched or unrelated (n = 120)	80 (66.7)				
Recipient CMV status					
Seronegative (n = 42)	7 (16.7)	**<.001**	**10.3 (4.39-24.1)**		
Seropositive (n = 263)	177 (67.3)				
Donor CMV status					
Seronegative (n = 81)	46 (56.8)	.438	1.23 (.73-2.05)		
Seropositive (n = 222)	137 (61.7)				
Donor/recipient CMV status					
D-/R- (n = 21)	3 (14.3)	**<.001**‡	**7.64 (3.51-16.6)**	**<.001**‡	**10.5 (4.35-25.4)**
D+/R- (n = 22)	6 (27.3)				
D-/R+ (n = 60)	43 (71.7)				
D+/R+ (n = 200)	131 (65.5)				
GVHD					
Absent (n = 75)	40 (53.3)	.127	1.51 (.89-2.56)	.127	1.62 (.87-3.03)
Present (n = 226)	143 (63.3)				
Chronic (n = 66)	37 (56.1)	.148	1.53 (.86-2.76)		
Acute (n = 160)	106 (66.3)				
Acute grade <2 (n = 42)	26 (61.9)	.561	1.25 (.59-2.65)		
Acute grade ≥2 (n = 97)	65 (67.0)				

* Univariate analysis.
† Multivariate logistic regression analysis.
‡ The analysis was performed considering D-/R-, D+/R-, and D-/R+ versus D+/R+.

serostatus, we observed a significant difference in the distribution ($P < .001$); nevertheless, we observed that there were no differences between D-/R- and D+/R- ($P = .252$) and between D-/R+ and D+/R+ ($P = .373$). In fact, we observed a significant difference when comparing D-/R- and D+/R- versus D-/R+ and D+/R+ ($P \leq .001$; OR, 7.64; 95% CI, 3.51 to 16.6). Moreover, and despite not being statistically significant, the prevalence of CMV infection tended to be higher among mismatched or unrelated donors (67.0% versus 56.0%, respectively) ($P = .068$; OR, 1.56; 95% CI, .97 to 2.51).

The multivariate logistic regression adjusting the analysis for covariates revealed an over 10-fold increased risk for CMV reactivation for D-/R+ and D+/R+ patients ($P < .001$; OR, 10.5; 95% CI, 4.35 to 25.4) and aHSCT with mismatched or unrelated donors ($P = .004$; OR, 2.54; 95% CI, 1.34 to 4.80).

Kinetics of CMV Infection

Considering the TTI, we observed that 166 patients (54.4%) in our cohort developed early CMV infection (≤100 days after transplantation) and only 18 patients (5.9%) developed late CMV infection (>100 days after transplantation). In our series, the median TTI was 29 days (range, 0 to 1285) and the median DOI was 10 days (range, 2 to 372).

Univariate and multivariate analyses were used to characterize the clinical variables that were associated with time-dependent characteristic of CMV infection (Table 4).

Cox regression analysis showed that the median TTI was significantly longer in patients >38 years old (36.0 versus 27.0 days, $P = .02$), those receiving peripheral blood as the source of graft (33.0 versus 23.5 days, $P = .001$), those undergoing RIC (34.5 versus 28.0 days, $P = .009$), those with related donors (40.0 versus 22.0 days, $P < .001$), and those with CMV-seropositive donors (33.0 versus 24.0 days, $P = .012$). However, when performing multivariate analysis, combining all information, the results showed that TTI was only associated with donor/HLA status ($P < .001$).

In addition, we observed that the median DOI was significantly longer in patients ≤ 38 years of age (11.0 versus 7.0 days, $P = .017$), those receiving cord blood or bone marrow transplants (20.5 versus 8.0 days, $P = .011$), those with mismatched or unrelated donors (20.0 versus 7.0 days, $P < .001$), and those with CMV-seronegative donors (14.0 versus 8.0 days, $P < .001$). When performing multivariate analysis, the results showed that DOI was only associated with donor/HLA status ($P < .001$) and with CMV D+/R+ ($P = .029$).

Patient Outcomes

The median time of hospitalization was 29 days (range, 9 to 395) and the overall median follow-up time after transplantation of our case series was 22 months (range, 0 to 68). Regarding the success of engraftment, we observed that the 291 patients had neutrophil counts $>.5 \times 10^9$/L, whereas only

Table 4
Analysis of CMV Infection Kinetics among aHSCT Recipients

	TTI, Median (IQR), d	*P* Value*	DOI, Median (IQR), d	*P* Value*
Gender				
Female (n = 72)	28.5 (19.0-47.0)	.360	9.5 (2.0-21.0)	.764
Male (n = 112)	32.0 (22.0-49.0)		11.0 (2.0-28.0)	
Age				
≤38 (n = 88)	27.0 (18.0-40.0)	**.020**	11.0 (2.2-30.8)	**.017**
>38 (n = 96)	36.0 (23.5-54.8)		7.0 (2.0-20.0)	
Stem cell source				
CB or BM (n = 30)	23.5 (17.0-36.0)	**.001**	20.5 (7.8-52.2)	**.011**
PBSC (n = 154)	33.0 (22.0-50.5)		8.0 (2.0-21.0)	
Phase at transplantation				
RC1 (n = 115)	32.0 (21.0-49.0)	.810	10.0 (2.0-21.0)	.929
RC ≥ second or active (n = 54)	34.5 (22.0-52.5)		12.0 (2.0-20.2)	
Conditioning regimen				
Myeloablative (n = 110)	28.0 (18.0-41.3)	**.009**	10.0 (2.0-25.0)	.597
RIC (n = 74)	34.5 (23.8-60.0)		12.5 (2.0-24.0)	
Donor/HLA status				
Related (n = 104)	40.0 (27.0-60.0)	**<.001**	7.0 (2.0-15.0)	**<.001**
Mismatched or unrelated (n = 80)	22.0 (17.0-32.0)		20.0 (7.0-41.2)	
Recipient CMV status				
Seronegative (n = 7)	47.0 (18.0-54.0)	.603	17.0 (2.0-99.0)	.497
Seropositive (n = 177)	29.0 (21.0-47.0)		10.0 (2.0-24.0)	
Donor CMV status				
Seronegative (n = 44)	24.0 (18.0-39.5)	**.012**	14.0 (7.0-52.8)	**<.001**
Seropositive (n = 137)	33.0 (22.0-50.5)		8.0 (2.0-19.5)	
Donor/recipient CMV status				
D-/R-, D+/R- (n = 9)	46.0 (16.0-52.5)	.922	21.0 (2.5-110.5)	.198
D-/R+, D+/R+ (n = 174)	29.0 (21.0-47.0)		10.0 (2.0-21.5)	
GVHD				
Absent (n = 40)	28.0 (21.0-41.8)	.231	8.5 (2.0-24.8)	.634
Present (n = 143)	32.0 (21.0-49.0)		10.0 (2.0-23.0)	
Chronic (n = 37)	32.0 (25.0-41.5)	.208	6.0 (2.0-18.5)	.534
Acute (n = 106)	32.5 (18.8-56.0)		12.0 (3.0-25.0)	
Acute grade <2 (n = 26)	25.5 (18.8-40.0)	.056	8.0 (2.8-21.0)	.207
Acute grade ≥2 (n = 65)	38.0 (21.0-66.0)		14.0 (2.5-25.0)	

IQR indicates interquartile range.
* Cox regression analysis.

260 had platelet counts >20 × 10^9/L. The rate of total engraftment success was 85.2% (260 of 305). Univariate (OR, 3.78; 95% CI, 1.93 to 7.39; $P < .001$) and multivariate (OR, 4.08; 95% CI, 1.78 to 9.38; $P = .001$) analysis revealed that engraftment success was only significantly associated with a myeloablative regimen (data not shown). The median time to neutrophil engraftment was 14 days (range, 1 to 95) and median time to platelet engraftment was 13 days (range, 1 to 377). Statistical analysis showed no correlation between CMV infection and time to neutrophil ($P = .882$) or platelet ($P = .103$) engraftment.

Disease relapse was observed in 73 patients (23.9%) within a median of 5 months (range, 0 to 45). Univariate and multivariate Cox regression analyses were used to characterize the clinical variables that were associated with disease relapse (Table 5) and no statistical significant association was found. Nevertheless, the multivariate analysis revealed a tendency to increased risk of relapse associated with peripheral blood as the source of graft (OR, 7.84; $P = .071$) and with complete remission ≥ second or active disease at the time of transplantation (OR, 1.70; $P = .076$).

At the end of the follow-up period, 194 patients (63.6%) were still alive with a median follow-up of 37 months (range, 2 to 68). To characterize the variables potentially associated with mortality, both univariate and multivariate analyses were performed (Table 5). The univariate analysis revealed that the risk of death was significantly increased in patients who underwent aHSCT with peripheral blood (OR, 2.16; 95% CI, 1.13 to 4.13; $P = .020$), with mismatched or unrelated donor (OR, 2.48; 95% CI, 1.53 to 4.01; $P < .001$), who developed aGVHD (OR, 2.22; 95% CI, 1.22 to 4.04; $P = .009$), or who developed CMV infection (OR, 1.90; 95% CI, 1.26 to 2.87; $P = .002$). In addition, the multivariate Cox regression analysis confirmed that the risk of death was significantly increased in patients with CMV infection (OR, 1.76; 95% CI, 1.07 to 2.90; $P = .025$), older than 39 years (OR, 1.89; 95% CI, 1.14 to 3.12; $P = .013$), submitted to aHSCT with peripheral blood (OR, 3.02; 95% CI, 1.33 to 6.86; $P = .008$), or with mismatched or unrelated donor (OR, 2.16; 95% CI, 1.48 to 3.13; $P < .001$). On the other hand, patients who developed GVHD have a significant decreased risk of death (OR, .47; 95% CI, .30 to .75; $P = .002$).

Overall, in our case series, we observed that patients who developed CMV infection had significantly reduced post-transplantation survival (median, 16.0 versus 36.0 months; $P = .002$) (Figure 1).

DISCUSSION

Viral infections remain 1 of the most important complications after aHSCT, with different impacts, depending on the moment of acquisition: infections caused by herpes simplex virus and hepatitis B and C viruses appear usually within the first month after transplantation, CMV is frequent between the first and fourth month after transplantation, and other latent viruses such as varicella-zoster virus and Epstein-Barr virus appear mainly between the second and sixth months after transplantation [4,15,22,23].

In this study, we intended to characterize the occurrence of CMV infection in 305 consecutive and unselected Portuguese patients undergoing aHSCT at the Portuguese

Table 5
Analysis of Disease Relapse and Mortality among aHSCT Recipients

	Relapse					Mortality				
	n (%)	*P* Value*	HR* (95% CI)	*P* Value†	HR† (95% CI)	n (%)	*P* Value*	HR* (95% CI)	*P* Value†	HR† (95% CI)
Gender										
Female (n = 119)	28 (23.5)	.472	.84 (.51-1.38)	.172	.66 (.37-1.20)	42 (35.3)	.750	1.06 (.72-1.56)	.676	.92 (.60-1.39)
Male (n = 186)	45 (24.2)					69 (37.1)				
Age										
≤38 (n = 153)	37 (24.2)	.302	1.36 (.85-2.19)	.199	1.43 (.83-2.45)	51 (33.3)	.250	1.24 (.86-1.81)	**.013**	**1.89 (1.14-3.12)**
>38 (n = 152)	36 (23.7)					60 (39.5)				
Stem cell source										
CB or BM (n = 48)	3 (6.3)	.230	2.43 (.57-10.3)	.071	7.84 (.84-73.1)	10 (20.8)	**.020**	**2.16 (1.13-4.13)**	**.008**	**3.02 (1.33-6.86)**
PBSC (n = 257)	70 (27.2)					101 (39.3)				
Phase at transplantation										
RC1 (n = 199)	46 (23.1)	.075	1.60 (.95-2.68)	.076	1.70 (.95-3.04)	64 (32.2)	.582	1.13 (.73-1.74)	.687	.91 (.57-1.45)
RC ≥ second or active (n = 85)	23 (27.1)					30 (35.3)				
Conditioning regimen										
RIC (n = 120)	22 (18.3)	.694	1.11 (.67-1.84)	.533	1.23 (.65-2.32)	39 (32.5)	.260	1.25 (.85-1.85)	.347	1.27 (.77-2.11)
Myeloablative (n = 185)	51 (27.6)					72 (38.9)				
Donor										
Related (n = 185)	48 (25.9)	.266	1.33 (.81-2.20)	.146	1.58 (.85-2.95)	52 (28.1)	**<.001**	**2.48 (1.53-4.01)**	**<.001**	**2.16 (1.48-3.13)**
MUR (n = 120)	25 (20.8)					59 (49.2)				
Recipient CMV status										
Seronegative (n = 42)	5 (11.9)	.661	1.23 (.49-3.07)			12 (28.6)	.332	1.35 (.74-2.47)		
Seropositive (n = 263)	68 (25.9)					99 (37.6)				
Donor CMV status										
Seronegative (n = 81)	20 (24.7)	.586	1.16 (.68-1.96)			36 (44.4)	.104	.72 (.48-1.07)		
Seropositive (n = 222)	53 (23.9)					74 (33.3)				
Donor/recipient CMV status										
D-/R-, D+/R- (n = 43)	5 (11.9)	.661	1.23 (.49-3.07)	.868	1.09 (.40-2.96)	12 (27.9)	.264	1.41 (.77-2.56)	.752	1.12 (.55-2.26)
D-/R+, D+/R+(n = 260)	68 (26.2)					98 (37.7)				
GVHD										
Absent (n = 75)	24 (32.0)	.233	.74 (.45-1.22)	.263	.74 (.43-1.26)	32 (42.7)	.124	.72 (.47-1.09)	**.002**	**.47 (.30-.75)**
Present (n = 226)	49 (21.7)					75 (33.2)				
Chronic (n = 66)	14 (21.2)	.207	1.50 (.80-2.83)			13 (19.7)	**.009**	**2.22 (1.22-4.04)**		
Acute (n = 160)	35 (21.9)					62 (38.8)				
Acute grade <2 (n = 42)	8 (19.0)	.888	1.06 (.45-2.54)			11 (26.2)	.182	1.58 (.81-3.07)		
Acute grade ≥2 (n = 97)	21 (21.6)					40 (41.2)				
CMV infection										
Negative (n = 121)	29 (24.0)	.145	1.43 (.88-2.32)	.891	.96 (.54-1.71)	32 (26.4)	**.002**	**1.90 (1.26-2.87)**	**.025**	**1.76 (1.07-2.90)**
Positive (n = 184)	44 (23.9)					79 (42.9)				

HR indicates hazard ratio; MUR, mismatched or unrelated.
* Univariate analysis.
† Multivariate Cox regression analysis.

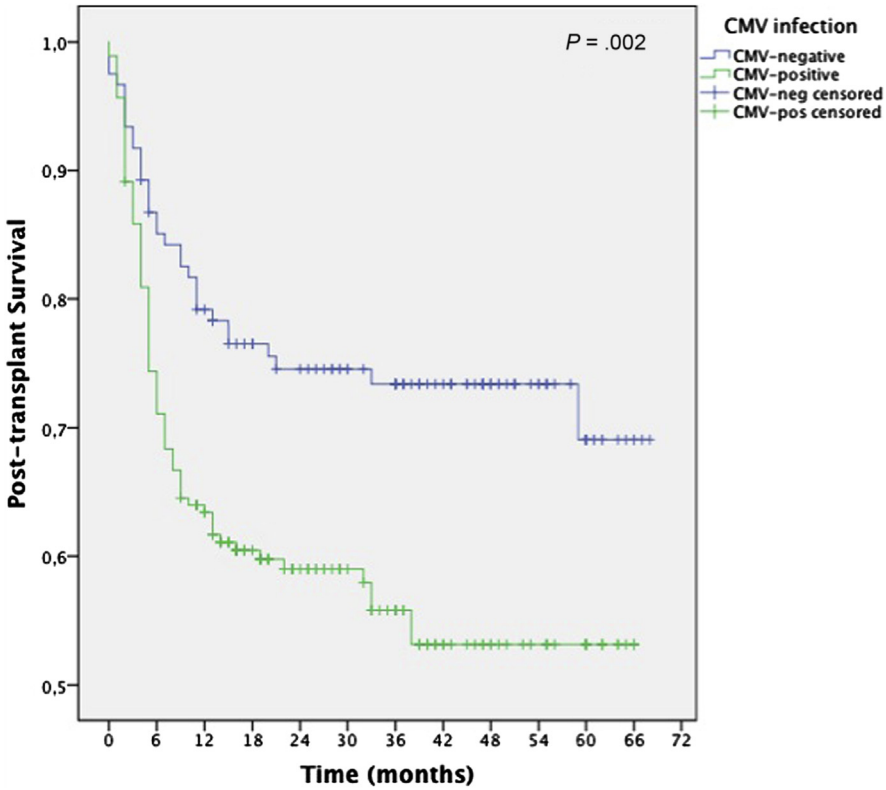

Figure 1. Association between CMV infection and post-transplantation survival in aHSCT patients. Kaplan-Meier plots with log-rank test estimate the post-transplantation survival of aHSCT patients with and without CMV infection.

Institute of Oncology of Porto. These data have not been reported previously, and although limited by its retrospective design, the study was able to include all patients submitted to aHSCT over a 5-year period. Regarding our case series, we observed that the majority of patients were male adults with a diagnosis of acute leukemia. The majority of aHSCT was performed with peripheral blood from related donors and a myeloablative regimen was used as the most frequent conditioning regimen. The rate of successful engraftment was 85.2%, disease relapse was observed only in 23.9% of patients, and overall mortality was 37.4%. Moreover, the follow-up period was very long for the majority of patients, considering that, in our case series, the majority of CMV reactivation occurred during the first 100 days after transplantation or within the first year [18,24].

Our results showed that the overall prevalence of CMV infection in aHSCT patients was 60.3%. These data point to a relatively high prevalence of CMV infection in our series. Nevertheless, the prevalence of CMV infection in aHSCT differs from study to study according to some population specificities: source of graft, age of patients, type of underlying disease, and conditioning regimen [25-28]. Despite some differences in the frequencies, we observed that CMV infection was not associated with gender, age at transplantation, underlying disease, stem cell source, phase at transplantation, conditioning regimen, and donor CMV seropositivity (see also supplementary table). Some authors state that CMV infection is more frequent in peripheral blood stem cells (PBSC) graft recipients rather than in cord blood or bone marrow recipients [29-32], although these data do not seem to be clear, as some authors state no difference [33]. Moreover, the use of RIC has been also described as correlated with increased susceptibility to CMV infection, as patients were more prone to viral reactivation in neutrophils. Nevertheless, the data analysis revealed that CMV infection was significantly more common in CMV D-/R+ and D+/R+ patients (66.9% versus 20.9%; OR, 7.64) and in mismatched or unrelated donors (66.7% versus 56.2%; OR, 2.21). Risk factors for CMV infection after aHSCT have been widely studied and the majority of studies showed that CMV seropositivity and mismatched or unrelated donors are consistently associated with increased risk of reactivation [11,21,27,34-38]. It has been suggested that there is a significant increase of immunosuppression in patients with mismatched or unrelated donors and, therefore, the reactivation of CMV is expected to be more frequent [39]. Hence, these data reinforce the need of implementation of effective prophylactic or preemptive measures with a correct assessment of the risk of CMV reactivation for each patient [11,40-42].

Literature states that CMV reactivation occurs mainly in the first 100 days after transplantation [18,24,27,30,34], and in our study we observed that the majority of patients developed CMV infection during this period (90.2% versus 9.8%). In our series, the median TTI was 29 days, which is consistent with other reports that show similar results, supporting the evidence that CMV reactivation is more frequent during the period of higher immunosuppression [18,27,39]. In addition, we have found that patients $\leq$38 years old who underwent myeloablative regimen with cord blood or bone marrow grafts from mismatched or unrelated CMV-seronegative donors had reduced TTI. Nevertheless, the use of different methodologies for CMV detection may have a great impact on the sensitivity of diagnosis and, therefore,

have an impact on the efficiency of treatment; however, intra- and interlaboratory variability has been challenging clinicians [11,43,44]. In our institution, the detection of CMV is mainly based on the pp65 antigenemia, with PCR detection only performed in cases where pp65 antigenemia is not possible. Nevertheless, several reports have showed that molecular techniques are more sensitive and, therefore, can detect viral reactivation earlier than pp65 antigenemia [42,44,45]. There has been a large discussion regarding the best approach for CMV detection because its utility has an impact on preemptive treatment, and the major problem is still the definition of a clinical cut-off value for the implementation of preemptive treatment [42,44,46]. Recently, Cardenoso et al. have shown a relevant correlation of pp65 antigenemia with CMV viral load, which requires more studies to evaluate the intra- and inter-laboratory performance [44].

In our case series, we observed that the median DOI was 10 days and that it was more prolonged in patients $\leq$38 years old who underwent transplantation with cord blood or bone marrow grafts from mismatched or unrelated CMV-seronegative donors. In fact, CMV infection is thought to be more complicated in some patients and, therefore, a correct treatment approach is required. In our institution, preemptive strategy for CMV disease prevention is preferred to prophylactic approach as it has been shown that the use of prophylactic antiviral drugs is associated with a delay in the recovery of the cellular immune response critical to the control of viral infection and, therefore, might increase the risk of infection in some patients [18,24]. Moreover, there is a discussion of which is the best approach for the prevention of CMV reactivation: acyclovir/valacyclovir or GCV/VGCV. Although literature indicates that the most effective approach seems to be the use of intravenous GCV [11], in our institution, oral VGCV is the preferred drug to avoid some of the severe consequences of GCV (myelotoxicity and renal toxicity), whereas GCV is reserved for cases of gastrointestinal intolerance. In fact, VGCV, the oral prodrug of GCV, has been shown to be efficient in preemptive treatment of CMV infections in aHSCT patients without significant toxicity [47]. These differences in either prophylactic or preemptive approach and first-line treatment choice reinforce the need of guidelines for patients undergoing HSCT, similar to those that have been reported for solid organ transplants recipients [48,49].

Our study revealed that post-HSCT disease relapse was present in 23.9% of cases. In fact, disease relapse is a relatively frequent event, ranging from 30% to 70%, and is dependent on the several factors, including disease status at the time of transplantation, donor source, and conditioning regimen [50-52]. Recently, a study from Elmaagacli et al. has shown that CMV reactivation was associated with reduced risk of relapse in patients with acute myeloid leukemia [53] and actually, these results were corroborated by a few authors who describe similar evidence [26,42,54]. In our study, we observed no association with disease relapse, except for a tendency regarding the use of peripheral blood as stem cell source or the presence of second complete remission or active disease at the time of transplantation. Moreover, we observed no significant differences regarding disease relapse and CMV infection according to the underlying disease (data not shown). The evidence in the literature has led to a great discussion regarding the possibility that CMV infection could have an antileukemic effect by infecting leukemic cells, and that still must be elucidated [55].

GVHD is also a significant cause of morbidity and mortality in aHSCT patients and, in fact, CMV infection seems to be frequently found concomitantly with the presence of gastrointestinal GVHD [21,56]. Nevertheless, in our case series, we found no significant association of CMV infection with GVHD development. Despite the fact that GVHD was a frequent event observed in our case series, with a total of 226 patients developing either aGVHD or cGVHD, it was more frequent in patients receiving transplants of PBSC with myeloablative conditioning. In fact, the literature indicates that transplants of PBSC are associated with increased risks of both severe aGVHD and cGVHD [57,58]. Moreover, it has been widely described that RIC regimens allow a translation from host to donor immune system without the development of GVHD, contrary to myeloablative regimens [59,60]. Despite the existent consensus regarding some of the GVHD predisposing factors, we are still lacking guidelines for the standardization of risk assessment and therapeutic strategy [57].

Although pneumonia and gastrointestinal disease are the most frequent clinical manifestations in aHSCT recipients, CMV infection is highly associated with mortality [8-10,21,61]. In agreement with literature, our case series showed that patients who developed CMV infection had a significantly reduced median post-transplantation survival (16.0 versus 36.0 months). In the past 20 years, several studies stated that CMV infection is consistently associated with increased morbidity and mortality in aHSCT patients [10,11,21]. In fact, we verified that in our case series, the risk of death in patients with CMV infection is increased 2-fold compared with those without CMV infection.

To the best of our knowledge, this is the first study reporting CMV infection among aHSCT recipients in Portugal. By performing a retrospective review at the Portuguese Institute of Oncology of Porto between 2008 and 2012, we have included data from a large cohort of patients who underwent aHSCT. Our study revealed that CMV infection was a frequent event, especially in CMV-seropositive recipients and patients with mismatched or unrelated donors. Moreover, we have identified several factors that affect the median TTI and DOI of CMV infection, and, therefore, we now have important data that should be used to select patients who will benefit from specific prophylactic or preemptive strategies. Finally, as CMV infection was revealed to be highly correlated with mortality of aHSCT patients, it is extremely important to increase attention to the selection of more sensitive CMV detection methods and better treatment options to avoid CMV-associated morbidity and mortality.

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SUPPLEMENTARY DATA

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