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Impact of Anti-CMV IgG Titers and CD34 Count Prior to Hematopoietic Stem Cell Transplantation from Alternative Donors on CMV reactivation

Leonardo Javier Arcuri[1,*], Marcelo Schirmer[1], Marta Colares[1], Simone Maradei[1], Rita Tavares[1], Maria Claudia Rodrigues Moreira[1], Renato de Castro Araujo[1], Decio Lerner[1], Antonio Guilherme Fonseca Pacheco[2]

[1] *Instituto Nacional de Cancer, Centro de Transplante de Medula Óssea, Rio de Janeiro, Brazil*
[2] *Fundação Oswaldo Cruz, Programa de Computação Científica, Rio de Janeiro, Brazil*

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A B S T R A C T

Cytomegalovirus (CMV) reactivation remains one of the main infectious complications following hematopoietic stem cell transplantation (HSCT). In this study, we explored the role of anti-CMV antibody titers in HSCT from alternative donors and to compare the risk of CMV reactivation between posttransplant cyclophosphamide-based haploidentical HSCT and antithymocyte globulin-based unrelated donor (URD) HSCT. We included 98 CMV-positive patients, 30 undergoing haploidentical HSCT and 68 undergoing URD HSCT. The majority of patients had a malignant disease (84%), received a myeloablative conditioning regimen (78%), and received a bone marrow graft (90%). The median pretransplantation anti-CMV IgG level was 109 U/mL. With median follow-up of 2.2 years, a total of 72 CMV reactivations occurred in 50 patients. There was no difference in CMV reactivation pattern between haploidentical HSCT recipients and URD HSCT recipients. In multivariable analysis until the first event, the incidence of CMV reactivation was higher in patients with anti-CMV IgG levels >100 U/mL (hazard ratio [HR], 2.38; P = .005) and in patients diagnosed with grade II-IV acute graft-versus-host disease (GVHD) (HR, 10.8; P = .003) after day +50 and lower in patients who received higher doses of CD34 cells (HR, .44; P = .006). In multivariable analysis for recurring events, the incidence of CMV reactivation was higher in patients receiving reduced-intensity conditioning (HR, 1.69: P = .04) and in patients with acute GVHD (HR, 1.88; P = .02), and lower in those who received higher doses of CD34 cells (HR, .55; P = .01). In summary, we have shown that pretransplantation anti-CMV IgG titers are correlated with CMV reactivation risk. More studies are needed to assess how this information can be incorporated in HSCT. The use of high-dose cellular grafts, a modifiable risk factor, also protects against CMV reactivation.

INTRODUCTION

Cytomegalovirus (CMV) reactivation remains one of the main infectious complications following hematopoietic stem cell transplantation (HSCT). Monitoring for CMV reactivation by either PCR or pp65 antigenemia and instituting early treatment have led to a decrease in CMV disease incidence from 24% to 30% to 5% to 6%. Likewise, CMV mortality has been reduced from 20% to 0 to 2% [1,2]. Nevertheless, CMV reactivation is quite frequent, and its treatment—ganciclovir—carries a substantial risk of toxicity, and thus CMV remains a major source of morbidity post-HSCT [1-4].

The interactions of CMV with the immune system are quite complex, altering the expression of membrane proteins (including HLA) and cytokine production, which may explain in part why patients who reactivate CMV are at increased risk of bacterial and fungal infections [4] and have a lower risk of acute leukemia relapse [5].

CMV control relies on cellular immune response, with the role of humoral immunity believed to be marginal. Multiple reactivations and multiple antiviral exposure may lead to ganciclovir-resistant infections, which are associated with higher mortality and morbidity [4,6-9]. Mutations of the viral protein kinase *UL97* and viral DNA polymerase *UL54* genes are the 2 most important causes of ganciclovir resistance [6]. Established antiviral agents for CMV-resistant infections are foscarnet and cidofovir [10], neither of which is available in Brazil.

Bruminhent et al [11] have shown that anti-CMV titers ≥60 AU/mL protect against CMV reactivation following liver transplantation. This effect was more pronounced in the

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*Correspondence and reprint requests: Leonardo Javier Arcuri, Guaramomis 480/64, Planalto Paulista, São Paulo, SP CEP 04076-010, Brazil.
E-mail address: leonardojavier@gmail.com (L.J. Arcuri).
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subgroup of patients receiving grafts from CMV-positive donors (hazard ratio [HR], 2.2; $P = .02$). In a retrospective study reported by Goldstein et al [12], patients undergoing sibling donor HSCT who did not receive prophylactic immunoglobulin were at greater risk of CMV reactivation compared with those who did receive it (44% versus 13%; $P = .001$). In summary, both studies suggest a contribution of humoral immunity in CMV reactivation.

The objectives of the present study were to explore the role of anti-CMV antibody titers in HSCT and to compare the risk of CMV reactivation in post-transplantation cyclophosphamide (PTCy)-based haploidentical (haplo) HSCT and antithymocyte globulin (ATG)-based unrelated donor (URD) HSCT.

METHODS

We retrospectively analyzed 98 CMV-positive patients with hematologic malignancies or nonmalignant diseases who underwent HSCT from alternative donors between 2015 and 2020. Anti-CMV antibodies were quantified before HSCT using a chemiluminescence immunoassay with the DiaSorin LIAISON Kit (Saluggia, Italy). All CMV reactivations were analyzed using a Cox model for recurrent events. For comparison, we also ran a Cox model until the first reactivation. Cox models were selected based on the lowest Akaike information criterion AIC, using a backward-forward algorithm. Graft-versus-host disease (GVHD) was included as a time-dependent covariate. Proportional hazards assumptions were checked graphically, using Schoenfeld residuals. We dealt with a nonproportional hazard variable by adding an interaction term with time. Cumulative incidence curves were compared with Gray's test. All patients received acyclovir 500 mg/m^{2}3 times a day until marrow recovery and 250 mg/m^{2}twice daily thereafter. Any positive CMV pp65 antigenemia or any quantitative real-time PCR result >100 copies/mL was considered a CMV reactivation. These values are the cutoffs currently used in our service to start preemptive ganciclovir in HSCT patients from alternative donors. Patients were then considered off-risk for at least 14 days, or until a negative antigenemia or PCR result was recorded. Because CMV serostatus was not available for most donors, we performed a sensitivity simulation analysis. CMV positivity in unrelated donors was 85%; thus, we assumed a 85% probability of being CMV positive in those patients without this information, and performed 10,000 simulations, imputing missing CMV status, and 10,000 Cox models. All analyses and simulations were performed using R version 3.5.1 (R Project for Statistical Computing, Vienna, Austria).

RESULTS

Patient baseline characteristics are summarized in Table 1. In brief, a total of 68 URD HSCT recipients and 30 haplo-HSCT recipients treated for a hematologic malignancy (84%) or nonmalignant disease (16%) were included. One patient underwent an initial URD HSCT, followed by a haplo-HSCT after a relapse. Another patient underwent 2 HSCTs from 2 different URDs. The median patient age was 21 years (range, 3 to 68 years), and the cohort was 67% male. Grafts were bone marrow (BM) in 90% and mobilized peripheral blood stem cells (PBSCs) in 10%. A myeloablative conditioning (MAC) regimen was used in 78% of the patients, and a reduced-intensity conditioning (RIC) regimen was used in the remaining 22%. All URD HSCT recipients received ATG-based GVHD prophylaxis, and all haplo-HSCT recipients received PTCy-based prophylaxis. All patients were CMV-positive. Donor serostatus was available for 63 donors, and 87% were positive. The median pretransplantation anti-CMV IgG level was 109 AU/mL (Table 2).

With a median follow-up of 2.2 years, there were 72 CMV reactivations in 50 of the 98 patients (100-day cumulative incidence of 49%). The median time until first reactivation was 37 days (range, 8 to 440 days).

The incidence of CMV reactivation was higher in patients with anti-CMV IgG level >100 U/mL (100-day cumulative incidence, 63% versus 31%; $P = .004$) and in those who received a CD34 cell dose $\leq 1.6 \times$ 10E6/kg (62% versus 38%; $P = .006$) (Figure 1). The CD34 cell dose received was significant even when we included only patients who received BM grafts (62% versus 36%; $P = .01$).

Risk factors in multivariable analysis until first reactivation were anti-CMV IgG levels >100 U/mL (HR, 2.38; $P = .005$), CD34 cell dose $>1.6 \times$ 10E6/kg (HR, .44; $P = .006$) and acute GVHD grade II-IV after day +50 (HR, 10.8; $P = .003$). The factors identified in multivariable analysis in the recurrent events model were CD34 cell dose $>1.6 \times$ 10E6/kg (HR, .57; $P = .03$), acute GVHD grade II-IV (HR, 1.88; $P = .02$), and use of an RIC regimen (HR, 1.69; $P = .04$).

The type of donor was not predictive of CMV reactivation. The 100-day incidence of CMV reactivation was 45% for URD HSCT and 59% for haplo-HSCT ($P = .32$) (Figure 2). Two patients died of CMV pneumonitis, both in the unrelated group.

DISCUSSION

Our results suggest that anti-CMV IgG titers can predict CMV reactivation following HSCT from an alternative donor, either haploidentical or unrelated. Moreover, the risk was greater in patients who received smaller CD34 cell doses and in those with grade II-IV acute GVHD. However, our study failed to demonstrate a difference in the risk of CMV reactivation between haplo-HSCT with PTCy and URD HSCT with an ATG-based strategy.

Contrary to our expectations, the risk of CMV reactivation was significantly greater in patients with a pretransplantation anti-CMV IgG level >100 U/mL (HR, 2.38). To our knowledge, this finding has not been reported previously. We expected to find a lower risk of CMV reactivation in patients with higher anti-CMV IgG levels because (1) a significant beneficial effect of immunoglobulin has already been reported [12], (2) IgG has a long half-life, and (3) Bruminhent et al [11] reported a lower risk of CMV reactivation in patients with higher anti-CMV IgG titers in the liver transplantation setting. However, the results of Bruminhent et al suggest a significant role of liver graft infection in CMV reactivation following liver transplantation, whereas Pergam [13] and George et al [14] have shown that the transmission from a positive donor to a negative host is inefficient in HSCT, and that most CMV reactivations following HSCT arise from the recipient, and this difference could explain our results. It is important to emphasize that cellular response is actually considered the most critical factor for CMV control [15], and higher anti-CMV IgG levels might only reflect more frequent intermittent asymptomatic CMV reactivations and poorer CMV control, as shown by Parry et al [16]. CMV is a genetically diverse virus, which might influence virus growth [17], and patients with higher anti-CMV IgG levels may also carry a more active strain, making them more susceptible to reactivations following HSCT. An alternative explanation is that some effective cytotoxic T cells survive the conditioning regimen and help CMV control. The fact that the effect of the anti-CMV IgG levels were diluted in the Cox model for recurring events supports the latter hypothesis, because all recipient cytotoxic T cells eventually will be rejected by the donor cells.

We also found an association between infused CD34 cell dose and the risk of CMV reactivation. Most of the transplantations were performed using BM grafts, and this association remained significant when we analyzed only those patients. The effect was also present in the recurrent events model, suggesting that the infusion of higher CD34 cell doses induces faster immune reconstitution [18]. A higher CD34 cell dose in BM harvests has been shown to improve engraftment in haploidentical transplantation [19], improve survival following URD BM transplantation [20], increase neutrophil engraftment, and even reduce fungal infections [21,22]. However, to

Table 1
Patient Baseline Profile

Characteristic	Haplo-HSCT Recipients	URD HSCT Recipients, Donor CMV Not Missing	URD HSCT Recipients, Donor CMV missing	*P* Value	Total, Donor CMV Not Missing	Total, Donor CMV Missing
Total, n	30	33	35		63	35
Age				.318		
Yr, median (IQR)	22.5 (14.4-33.5)	22.5 (15.5-44.2)	18.4 (10.5-32.2)		22.5 (14.5-40.1)	18.4 (10.5-32.2)
Sex, n (%)				.594		
Male	21 (70)	20 (60.6)	25 (71.4)		41 (65.1)	25 (71.4)
Female	9 (30)	13 (39.4)	10 (28.6)		22 (34.9)	10 (28.6)
Donor sex, n (%)				.683		
Male	20 (66.7)	24 (72.7)	22 (62.9)		44 (69.8)	22 (62.9)
Female	10 (33.3)	9 (27.3)	13 (37.1)		19 (30.2)	13 (37.1)
CMV serostatus, n (%)				.824		
Positive	30 (100)	33 (100)	35 (100)		63 (100)	35 (100)
Donor CMV status, n (%)				<.001		
Negative	3 (10)	5 (15.2)	0 (0)		8 (12.7)	0 (0)
Positive	27 (90)	28 (84.8)	0 (0)		55 (87.3)	0 (0)
Missing	0 (0)	0 (0)	35 (100)		0 (0)	35 (100)
Disease, n (%)				.793		
Severe aplastic anemia	4 (13.3)	5 (15.2)	2 (5.7)		9 (14.3)	2 (5.7)
Fanconi anemia	0 (0)	1 (3)	1 (2.9)		1 (1.6)	1 (2.9)
PNH	0 (0)	1 (3)	0 (0)		1 (1.6)	0 (0)
ALL	12 (40)	11 (33.3)	17 (48.6)		23 (36.5)	17 (48.6)
AML	9 (30)	8 (24.2)	7 (20)		17 (27)	7 (20)
CML	1 (3.3)	5 (15.2)	3 (8.6)		6 (9.5)	3 (8.6)
JMML	0 (0)	0 (0)	1 (2.9)		0 (0)	1 (2.9)
Non-Hodgkin lymphoma	1 (3.3)	0 (0)	1 (2.9)		1 (1.6)	1 (2.9)
PRCA	0 (0)	0 (0)	1 (2.9)		0 (0)	1 (2.9)
Myelodysplastic syndrome	3 (10)	2 (6.1)	1 (2.9)		5 (7.9)	1 (2.9)
SWA	0 (0)	0 (0)	1 (2.9)		0 (0)	1 (2.9)
Sex match, n (%)				.34		
Other	23 (76.7)	29 (87.9)	26 (74.3)		52 (82.5)	26 (74.3)
Female to male	7 (23.3)	4 (12.1)	9 (25.7)		11 (17.5)	9 (25.7)
Disease Risk Index, n (%)				.947		
Nonmalignant	4 (13.3)	7 (21.2)	5 (14.3)		11 (17.5)	5 (14.3)
Low	2 (6.7)	3 (9.1)	3 (8.6)		5 (7.9)	3 (8.6)
Intermediate	15 (50)	14 (42.4)	16 (45.7)		29 (46)	16 (45.7)
High	7 (23.3)	6 (18.2)	10 (28.6)		13 (20.6)	10 (28.6)
Very high	2 (6.7)	3 (9.1)	1 (2.9)		5 (7.9)	1 (2.9)
HLA match, n (%)				<.001		
8/8	0 (0)	24 (72.7)	26 (74.3)		24 (38.1)	26 (74.3)
7/8	0 (0)	9 (27.3)	9 (25.7)		9 (14.3)	9 (25.7)
Haploidentical	30 (100)	0 (0)	0 (0)		30 (47.6)	0 (0)
Stem cell source, n (%)				.339		
BM	29 (96.7)	29 (87.9)	30 (85.7)		58 (92.1)	30 (85.7)
PBSCs	1 (3.3)	4 (12.1)	5 (14.3)		5 (7.9)	5 (14.3)
Conditioning regimen, n (%)				.017		
MAC	18 (60)	27 (81.8)	31 (88.6)		45 (71.4)	31 (88.6)
RIC	12 (40)	6 (18.2)	4 (11.4)		18 (28.6)	4 (11.4)
Anti-CMV IgG titer				.223		
AU/mL, median (IQR)	134.8 (93.4-387.7)	94.4 (41.6-147.7)	95.9 (36.1-242.1)		116 (55.9-180)	95.9 (36.1-242.1)
CD34 cell dose				.202		
median (IQR)	2.9 (1.8-3.8)	1.7 (1.3-3.2)	2 (1.2-3.1)		2.1 (1.4-3.4)	2 (1.2-3.1)

PNH, paroxysmal nocturnal hemoglobinuria; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; JMML, juvenile myelomonocytic leukemia; PRCA, pure red cell aplasia; SWA, Shwachman-Diamond anemia.

our knowledge, the impact of CD34 cell dose on the risk of CMV reactivation has not been reported previously.

The association identified between RIC and CMV reactivation suggests that the immunosuppression effects of RIC regimens are long-lasting. This has been reported previously in a cohort of URDs and matched-sibling donors [14]. All the RIC regimens were fludarabine-based (compared with only 35% of MAC regimens), and fludarabine has a long half-life. If

Table 2
Cox Models

Variables	Until First Reactivation	*P* Value	Recurring Events	*P* Value
Univariable analysis				
Age (each yr)	1.01 (1.00-1.03)	.12	1.01 (1.00-1.03)	.07
Female vs male	.79 (.44-1.43)	.44	.87 (.48-1.55)	.63
Anti-CMV IgG >100 UI/mL	2.48 (1.37-4.48)	.003*	1.92 (1.03-3.58)	.04*
Nonmalignant vs malignant	1.06 (.55-2.05)	.87	1.20 (.61-2.37)	.60
Haploidentical vs URD	1.29 (.71-2.33)	.30	1.12 (.65-1.94)	.68
PBSCs vs BM	1.42 (.65-3.12)	.38	1.91 (.90-4.08)	.09
RIC vs MAC	1.50 (.82-2.74)	.18	1.77 (1.01-3.08)	.04*
TNC >3.5 × 10E8/kg†	.53 (.30-.96)	.03*	.64 (.35-1.15)	.13
CD34 >1.6 × 10E6/kg	.49 (.28-.86)	.01*	.49 (.29-.83)	.007*
aGVHD, grade II-IV	1.90 (1.09-3.30)	.02*	1.73 (.96-3.12)	.07
Multivariable analysis				
RIC vs MAC	–	–	1.69 (1.02-2.80)	.04*
Anti-CMV IgG >100 UI/mL	2.38 (1.29-4.37)	.005*	1.58 (.89-2.78)	.11
CD34 >1.6 × 10E6/kg	.44 (.25-.79)	.006*	.55 (.35-.88)	.01*
aGVHD, grade II-IV	–	–	1.88 (1.12-3.18)	.02*
Until day +50	1.33 (.65-2.75)	.44	–	–
After day +50	10.8 (2.27-51.4)	.003*	–	–

* $P < .05$.
† Only BM grafts included.

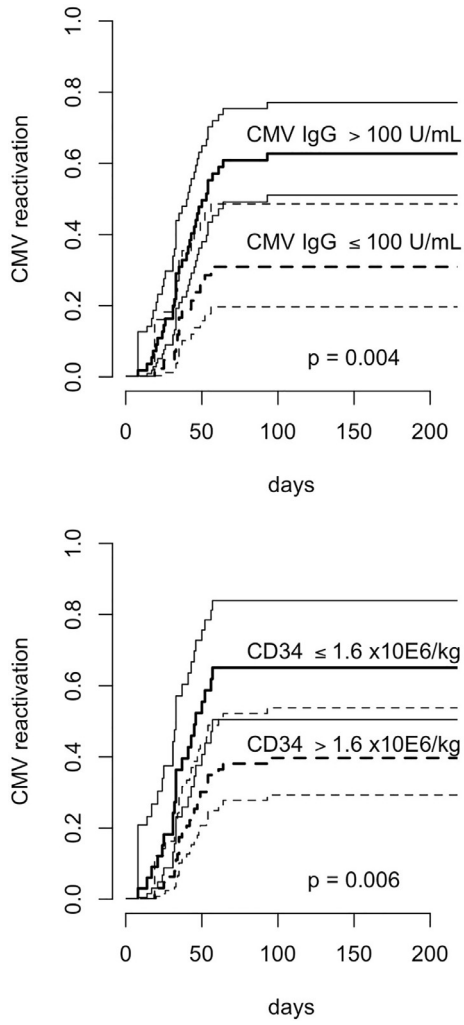

Figure 1. Cumulative incidence of CMV reactivation by anti-CMV levels (A) and CD34 dose (B). Bold curves represent cumulative incidence curves; light curves represent 95% CIs.

given until day -2, the infused graft is exposed to fludarabine. The association between conditioning regimen and risk of CMV reactivation was suggested only in the Cox model for recurring events. We believe that this model addresses the real problem following HSCT: multiple CMV reactivations. However, it came to our attention that none of the major randomized trials [23-28] used a recurring events model in their data analysis. Because it is well known that multiple CMV treatments lead to antiviral resistance and increased morbidity, we believe that analyzing CMV reactivation with a model for recurring events merits further explored.

As expected, CMV reactivation risk was higher in patients with grade II-IV acute GVHD. The cornerstone of GVHD treatment is increasing the immunosuppression, usually with corticosteroids. The net result was an increased risk of CMV reactivation.

We have not found different patterns of CMV reactivation between haploidentical transplantation with PTCy-based GVHD prophylaxis and URD with ATG-based prophylaxis. This is in agreement with previous reports [29,30]; however, this finding is not universal. Lin et al [31] found a higher CMV reactivation rate in haplo-HSCT compared with URD HSCT (86% versus 56%), and Duver et al [32] reported a higher incidence

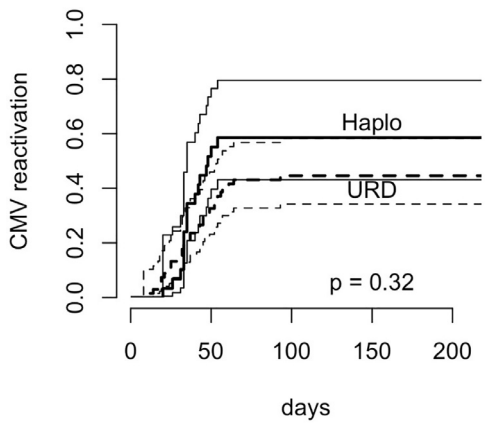

Figure 2. Cumulative incidence of CMV reactivation, by donor type. Bold lines indicate cumulative incidence curves; light lines represent 95% CIs.

of CMV reactivation with mismatched or haploidentical donors. Of note, in the study of Lin et al, haplo-HSCT recipients received dual in vivo T cell depletion GVHD prophylaxis with PTCy and ATG, and in the study of Duver et al, no haplo-HSCT recipients received PTCy.

The main limitation of our study is that CMV serostatus was not retrospectively available for all donors. In Brazil, proportions of CMV-positive donors and mothers >90% have already been reported [33,34], and in our study, 85% of URDs and 90% of haploidentical donors were CMV-positive among those with available results. Consequently, we ran 10,000 simulations using a 85% probability of a donor being CMV-positive and included this new information in the multivariable analysis. IgG titer was not a statistically significant risk factor in only .11% of the simulations, demonstrating a low probability of bias. We also performed the analysis only with patients with complete data. The HR for CMV titers in multivariable analysis was 2.09 (compared with the original 2.33). Importantly, donor CMV status was not associated with outcome (HR, 1.08; $P = .89$).

In summary, we have shown that low number of infused CD34 cells (a modifiable risk factor) was associated with CMV reactivation. In addition, pretransplantation anti-CMV IgG titer is associated with the risk of CMV reactivation. More studies are needed to explore how this finding can be incorporated into the CMV reactivation risk algorithm.

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