Regular manuscript

Regimen-related mucosal injury of the gut increased the incidence of CMV disease after allogeneic bone marrow transplantation.

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Abstract

Cytomegalovirus (CMV) infection is one of the major causes of morbidity in patients undergoing allogeneic stem cell transplantation (alloSCT). The incidences of CMV antigenemia and CMV disease in 43 patients who received allogeneic bone marrow transplantation (BMT) using a reduced-intensity conditioning (RIC) regimen, which mainly consisted of fludarabine, busulfan and total body irradiation, were compared with those in 68 patients who received a myeloablative conditioning (MAC) regimen, and risk factors for CMV antigenemia and CMV disease were identified.

Before engraftment, grade 3-4 mucosal injury due to the conditioning regimen was significantly decreased in RIC patients (stomatitis: P=0.02; diarrhea: P<0.01). Rate of engraftment, incidences of acute graft-versus-host disease (aGVHD) and rate of corticosteroid administration were not different in RIC patients and MAC patients.

Although the incidences of CMV antigenemia were not significantly different in RIC patients and MAC patients (64.1% vs. 57.8%, logrank, P=0.59), the incidence of CMV disease was significantly decreased in RIC patients (5.4% vs. 20.3%, logrank, P=0.04). CMV seropositivity in the patients (P<0.01) and corticosteroid administration (P<0.01) were revealed by multivariate analysis to be significant risk factors for CMV antigenemia. Grade II-IV aGVHD (P=0.02) and grade 3-4 diarrhea before engraftment (P=0.04) were revealed to be risk factors for CMV disease. The present study is the first study to show that severe diarrhea before engraftment is a significant risk factor for CMV disease. In summary, risk of CMV disease was significantly decreased in patients without severe mucosal injury of the gut due to the conditioning regimen before engraftment.

Keywords: reduced-intensity conditioning, bone marrow transplantation, cytomegalovirus infection, mucosal injury, graft-versus-host disease

Introduction

Cytomegalovirus (CMV) infection is one of the major causes of morbidity in patients undergoing allogeneic stem cell transplantation (allo-SCT). Preemptive antiviral therapy has been shown to reduce the risk of CMV disease [1-3]. Major risk factors for CMV infection are serological status of the donor and recipient, graft-versus-host disease (GVHD), corticosteroid administration and T-cell depletion [1,2,4-13]. Recently, reduced-intensity conditioning (RIC) regimens have been developed for patients who had been considered ineligible for SCT using a myeloablative conditioning (MAC) regimen because of advanced age or medical contraindications [14,15]. Although many studies have shown that infection before engraftment was reduced in patients undergoing RIC due to a shorter neutropenic period and less severe mucositis [16-19], risks of CMV infection have not been substantially reduced after RIC-SCT [1,7,8,10-12]. Again, we need to consider the difference in CMV infection depending on the RIC regimen because various RIC protocols have been developed and the toxicity profile might vary from one protocol to another due to variability in the degree of immunosuppression or myeloablation [8,10,11,14,15]. We should also consider the difference in CMV infection depending on the stem cell source [8,20].

The present study was a retrospective analysis to compare the incidence of CMV infection in 43 consecutive patients who received bone marrow transplantation (BMT) using an RIC regimen, which mainly consisted of fludarabine (FLU), busulfan (BU) and total body irradiation (TBI) (FLU/BU/TBI), in our institution with that in 68 patients who received MAC-BMT during the same period. The risk factors for CMV antigenemia and development of CMV disease were also investigated.

Patients and methods

Patients

One hundred eleven consecutive adult patients with advanced hematological diseases who received allogeneic BMT using RIC regimens (43 patients) or MAC regimens (68 patients) between September 2000 and March 2007 at Hokkaido University Hospital were analyzed for CMV infections. Twenty-eight patients received an RIC regimen due to advanced age (>50 years) and ten received an RIC regimen due to prior autologous transplantation (five patients overlapped with the patients of advanced age). A difference in the risk of CMV infection depending on stem cell

source has been reported [8, 20], and we cannot use PBSC from an unrelated donor (PBSC can be used only from related donors.) in Japan. Moreover, it has been reported that cord blood showed differences in the incidences of infections and kinetics of immunological recovery from other stem cell sources. Therefore, we analyzed only patients who received BMT. Patients who had already received allogeneic SCT were excluded from this study.

Conditioning regimens

In the RIC group, 38 (88.4%) of the patients received a conditioning regimen of FLU/BU/TBI, which consisted of FLU at a dose of 30 mg/m^2 once daily administered intravenously (i.v.) on days -7 to -2 (total dose: 180 mg/m^2) and BU at 1 mg/kg four times daily administered orally (p.o.) on days -3 and -2 (total dose: 8 mg/kg) combined with fractionated TBI at 2 Gy twice daily on day -1 (total dose: 4 Gy), and the other five patients received FLU plus melphalan (n=4) or FLU plus CY (n=1). In the MAC group, ten patients (14.7%) received a conditioning regimen of cyclophosphamide and TBI (CY/TBI), which consisted of CY at a dose of 60 mg/kg once daily administered i.v. on days -5 and -4 combined with fractionated TBI at 2 Gy twice daily on days -3 to -1 (total dose: 12 Gy), and 44 patients (64.7%) received CY/TBI plus VP-16 (VP/CY/TBI), in which VP-16 was added to CY/TBI at a dose of 15 mg/kg once daily administered i.v. on days -7 and -6 (total dose: 30 mg/kg) [21,22]. The other patients received other regimens of BU/CY or CY/TBI plus cytarabine. GVHD prophylaxis consisted of cyclosporine A and a short course of methotrexate (MTX, 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3 and 6) for HLA-matched related donor recipients, and tacrolimus plus a short course of MTX was given for HLA-matched unrelated donor or HLA-mismatched donor (MMD) recipients. The patients received GVHD prophylaxis from day -1 for 3 months and drug doses were tapered in patients with no active GVHD, the dose of cyclosporine A or tacrolimus being adjusted by plasma level.

Supportive care and infection prophylaxis

Levofloxacin (300 mg daily) was administered p.o. for prevention of bacterial infections until engraftment, and antifungals (fluconazole at 400 mg daily p.o., itraconazol capsules at 200 mg daily p.o. or micafungin at 100 mg daily i.v.) were administered for prevention of fungal infections. Oral acyclovir was given on day -7 to day 35 for prevention of herpes simplex virus infection. Oral trimethoprim-sulfamethoxazole or pentamidine inhalation was started after

engraftment for prevention of *Pneumocysts jiveroci* infection. Prophylactic intravenous immunoglobulin (10 g) was given biweekly until serum IgG levels reached >400mg/dL. Prednisolone was administered for patients who developed grade ≥2 acute GVHD (aGVHD) at a dose of 0.5-1.0 mg/kg daily according to a physician's decision. The dose of prednisolone administered was lower than the dose used in other countries because of the lower incidence of critical aGVHD in Japan [23].

CMV surveillance and treatment

Pretransplant serum samples from all patients and donors were tested for serologic evidence of past infection with CMV by an enzyme-linked immunosorbent assay or complement fixation test. When a patient and a donor were both negative for CMV, patients received CMV-negative blood products. When a patient or a donor was positive for CMV, the patient was given unscreened blood products. Surveillance blood CMV pp65 antigenemia was monitored once or twice a week between engraftment and day 100 post-SCT [1,10,24]. Patients with persistent CMV infection, GVHD and/or corticosteroid administration were screened beyond this period at the discretion of the doctor [1,3]. When a patient developed respiratory symptoms or abdominal symptoms suggestive of CMV disease, bronchoalveolar lavage or colonoscopy was performed to determine whether the patient had CMV disease. The patients received pre-emptive ganciclovir (GCV) at a dose of 5 mg/kg twice daily intravenously from onset day of CMV antigenemia until the second day of two consecutive days on which patients were negative for CMV antigenemia with clinical improvement. Patients with aGVHD received maintenance therapy of GCV (5 mg/kg once daily) for 2 weeks. Foscavir was administered for patients who showed rising CMV antigenemia, those who developed CMV disease despite GCV administration or those who developed serious toxicity due to GCV.

Definitions

CMV antigenemia was considered positive if there was more than 1 pp65+cell/ 10^4 neutrophils assessed. CMV pneumonitis was defined as the demonstration of CMV in tissue by culture or histology or in bronchoalveolar lavage (BAL) by culture, direct fluorescence antibody stain, or cytology in the presence of new or changing pulmonary infiltrates. However, detection of CMV by culture or by cytology showed low sensitivity and it was difficult to perform lung biopsy due to complications after SCT. Therefore, CMV pneumonitis was also defined as the demonstration of all of

following factors: detection of CMV by polymerase chain reaction (PCR), respiratory symptoms, presence of new or changing pulmonary infiltrates and CMV antigenemia. CMV enteritis was diagnosed when gastrointestinal signs or symptoms occurred, and evidence of CMV in the gastrointestinal tract was diagnosed by culture, immunohistochemistry, or in situ hybridization from biopsy specimens [10]. CMV hepatitis was diagnosed when liver dysfunction occurred, and evidence of CMV in liver tissue was diagnosed by culture, immunohistochemistry, or in situ hybridization from biopsy specimens. AGVHD and chronic GVHD (cGVHD) were graded by standard criteria [25,26]. Overall survival (OS) was calculated from the day of SCT until death or last follow-up.

End points and statistical analysis

The aims of this study were to compare the incidences of CMV antigenemia and the incidences of CMV disease in patients undergoing RIC and MAC regimens and to identify risk factors. Univariate analyses were performed using the χ^2 test and Fisher's exact test, as appropriate. The probabilities of CMV antigenemia, CMV disease, OS and PFS were estimated using the Kaplan–Meier method. Effects of the conditioning regimens on survival and CMV infections were studied using the logrank test. Multivariate logistic regression models were used to analyze the influence of selected variables with the forward stepwise method on the risk of CMV antigenemia and CMV disease. All *P*-values were two-sided and a *P*-value of 0.05 was used as the cutoff for statistical significance.

Results

Patient and transplantation characteristics

Patient and transplantation characteristics are summarized in Table 1. Median age, GVHD prophylaxis, underlying disease, prior autologous transplantation and months from diagnosis to transplantation were significantly different between RIC patients and MAC patients. CMV serostatus was not different between RIC patients and MAC patients.

Transplantation outcomes

Transplantation outcomes are summarized in Table 2. Stomatitis and diarrhea were assessed as regimen-related mucositis, and grade ≥3 stomatitis and grade ≥3 diarrhea were significantly less in RIC patients. Except for one patient who died early

after engraftment, all patients who achieved engraftment were assessed for aGVHD and CMV infection (RIC: n=39, MAC: n=65). Although incidence of aGVHD was not different between the groups, median onset day of aGVHD was significantly delayed in RIC patients [day 33 vs. day 20, $P<0.01$]. The rate of corticosteroid administration and the median dose of corticosteroid for GVHD (1 mg/kgprednisolone) were the same in RIC patients and MAC patients, but the median duration of corticosteroid therapy was longer in RIC patients than in MAC patients with marginal significance [RIC: median 180.5 days (range: 39 days – 975 days), MAC: 85 days (range: 20 days – 404 days), $P=0.08$]. The median follow-up period was 18.1 months (range: 0.1-83.4 months) for all patients and 30.3 months (range 8.1-83.4 months) for patients alive. The 2-year OS was not different between the groups. There was no difference between causes of death in RIC patients and MAC patients (RIC: disease progression, n=5; transplantation-related complication, n=8, vs. MAC: disease progression, n=13; transplantation-related complication, n=10).

CMV antigenemia and CMV disease (Table 2 and Figure 1)

Durations of CMV monitoring in RIC patients and MAC patients were similar. Incidences of CMV antigenemia were not different in RIC patients and MAC patients [RIC: n=25 (64.1%) vs. MAC: n=37 (57.8%), logrank, $P=0.59$], and the median onset day of CMV antigenemia was the same (day 43). Incidence of CMV disease was significantly decreased in RIC patients [RIC: n=2 (5.4%) vs. MAC: n=13 (20.3%), logrank, $P=0.04$, HR 0.24, 95% CI 0.1-1.0]. In the RIC group, 69.4% and 0.0% of the CMV-seropositive and CMV-seronegative patients, respectively, developed CMV antigenemia, and no difference was observed between RIC patients and MAC patients. One (2.7%) of the RIC patients developed CMV enteritis on day 45, and one (2.7%) of the RIC patients developed pneumonitis on day 185. In 11 patients who developed both aGVHD and CMV disease, nine patients (81.8%) developed aGVHD earlier than CMV disease. In MAC patients, eight (12.5%) of the patients developed enteritis, four (6.3%) of the patients developed pneumonitis and one (1.6%) of the patients developed hepatitis on median day of 47 (day 25-day 71). All cases of enteritis or hepatitis were diagnosed by tissue biopsy and immunohistochemical staining. No case was confirmed by culture. Only one patient who received an RIC regimen developed CMV disease beyond day 100. There was no difference in type of CMV disease between RIC patients and MAC patients ($P=0.2$ for enteritis, $P=0.7$ for pneumonitis). Only one patient who developed CMV pneumonitis following the MAC

regimen died of CMV infection.

Risk factors for CMV antigenemia and CMV disease (Table 3)

Univariate analysis showed that CMV seropositivity of patients at SCT, any grade of aGVHD, gastrointestinal aGVHD and corticosteroid administration were risk factors for CMV antigenemia. CMV seropositivity of the patients and corticosteroid administration remained significant in multivariate analysis.

Analyses of risk factors for CMV disease were also performed. In univariate analysis, MAC regimen, grade 3-4 diarrhea before engraftment, grade II-IV aGVHD, gastrointestinal aGVHD and corticosteroid administration were revealed to be significant risk factors for development of CMV disease. Bacterial infection and fungal infections occurred in 28% and 11% of the patients, respectively, and there were no differences in the incidences of CMV antigenemia (bacterial infection, *P*=0.12; fungal infection, *P*=0.91) and CMV diseases (bacterial infection, *P*=0.34; fungal infection, *P*=0.41) between patients with bacterial infection or fungal infection and patients without bacterial or fungal infection. Grade 3-4 diarrhea before engraftment and grade II-IV aGVHD remained significant in multivariate analysis. CMV disease did not occur in any of the patients who had grade 3-4 diarrhea before engraftment without grade II-IV aGVHD (Figure 2, Group C). However, in patients who developed grade II-IV aGVHD, the incidence of CMV disease was divided into two groups according to the presence of grade 3-4 diarrhea before engraftment [grade II-IV aGVHD+/grade 3-4 diarrhea+ (Group A): 46.7% vs. grade II-IV aGVHD+/grade 3-4 diarrhea- (Group B): 12.5%, logrank, *P*=0.01 HR 4.01 (1.38-18.54)]

Discussion

In this study, we analyzed the incidence and characteristics of CMV infections in patients who received allogeneic BMT after an RIC regimen of FLU/BU/TBI and we compared the incidences of CMV antigenemia and CMV disease in RIC patients and MAC patients.

CMV antigenemia developed in 64% of the RIC patients, a higher incidence than the incidence found in other studies (about 40-50%) [1,8,10,11,13]. It has been reported that the incidence of CMV activation was not reduced in RIC patients [1,7,8,10-12], and reported risk factors for CMV infection in RIC patients include CMV serostatus, HLA-matched unrelated donor, advanced age of donor, grade II-IV aGVHD, corticosteroid administration, Campath-1H and/or anti-thymocyte globulin in the

conditioning regimen and BM as a stem cell source [1,2,4-6,8-13,27,28]. In our study, all patients received BM as a stem cell source, and the number of high-risk patients of CMV serostatus in our study was larger than that in other studies, which might explain the higher incidence of CMV antigenemia. Multivariate analysis in this study confirmed CMV serostatus and corticosteroid administration as significant risk factors for CMV antigenemia.

Although the incidence of CMV antigenemia was not decreased in RIC patients, CMV disease was decreased in RIC patients. The incidence of CMV disease in this study was not increased compared to that in other studies and it was significantly decreased in RIC patients compared with that in MAC patients. In the present study, grade II-IV aGVHD and grade 3-4 diarrhea before engraftment were revealed by multivariate analysis to be significant risk factors for development of CMV disease. The incidences of grade II-IV aGVHD and the median dose of corticosteroid for GVHD in RIC patients and MAC patients were the same. The median duration of corticosteroid therapy was longer in RIC patients with marginal significance. Therefore, we do not think that the incidence of grade II-IV aGVHD and the intensity and the duration of immunosuppressants mainly contribute to the higher incidence of CMV disease in MAC patients. This is the first study to show an increased risk of CMV disease among patients with severe diarrhea due to the conditioning regimen, which reflected mucosal injury of the gut. Almost all of the CMV diseases that occurred in our patients were CMV enteritis; therefore, diarrhea was determined to be a risk factor for CMV disease. The incidence of CMV antigenemia was not different between patients with grade 3-4 diarrhea and patients without grade 3-4 diarrhea. Also, the incidences of grade II-IV aGVHD and gastrointestinal aGVHD were not different between patients with grade 3-4 diarrhea and patients without grade 3-4 diarrhea (data not shown). These findings suggested that severe diarrhea affected the incidence of CMV disease directly, not via aGVHD. CMV disease occurred in 46.7% of the patients who developed both grade 3-4 diarrhea and grade II-IV aGVHD, but CMV disease occurred in only 12.5% of the patients who developed grade II-IV aGVHD without grade 3-4 diarrhea. CMV disease occurred in only one patient who had no grade II-IV aGVHD. These results suggested that immunosuppression in the whole body due to aGVHD (and corticosteroid) was most important for “CMV reactivation” but that mucosal injury of the gut was also necessary for development of “CMV disease”. It has been reported that mucosal immunity of the gut is an important defensive system against pathogens including CMV and that immunoglobulin,

macrophages and T-lymphocytes play a pivotal role in this local immunity. Therefore, impairment of mucosal immunity of the gut due to the conditioning regimen might have influenced the incidence of CMV enteritis in our patients [29-31]. Risk of severe diarrhea before engraftment may be complicated with our MAC regimen, which mainly included VP-16 [22]. We should consider the increased risk for development of CMV disease in patients with severe diarrhea before engraftment and in these patients who develop grade II-IV aGVHD. Although our analysis has limitations due to its retrospective fashion and small sample size, patients who received an RIC regimen showed a lower risk of CMV disease, and mucosal injury of the gut was determined to be a significant risk factor for development of CMV disease. Further studies are needed to confirm these findings.

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Titles and legends to figures

Figure 1

Cumulative incidences of CMV antigenemia (a) and CMV disease (b).

Figure 2

Cumulative incidence of CMV disease according to the combination of two risk factors: grade II-IV aGVHD and grade 3-4 diarrhea before engraftment.

Group A included patients who had both grade II-IV aGVHD and grade 3-4 diarrhea before engraftment (n=15). Group B included patients who had grade II-IV aGVHD but did not develop grade 3-4 diarrhea before engraftment (n=32). Group C included patients who did not develop grade II-IV aGVHD (n=52).

Table 1: Patients and transplantation characteristics

	RIC (n=43)	MAC (n=68)	*P-value*
Age, median (range)	52 (17-66)	34.5 (15-58)	*<0.01*
Patient sex, %			
Male	48.8%	63.2%	*0.13*
Underlying disease, %			
Acute leukemia	16.3%	54.4%	*<0.01*
MDS	27.9%	10.3%	
CML	9.3%	20.6%	
ML/ATL	27.9%	10.3%	
MM	11.6%	0.0%	
Others	7.0%	4.4%	
Prior autologous SCT, %	23.3%	0.0%	*<0.01*
Diagnosis to SCT, months; median (ran	22.5 (1.7-240)	8.3 (5.0-276.1)	*<0.01*
CR at SCT, %	53.5%	68.7%	*0.11*
Donor, %			
MRD	23.3%	30.9%	*0.48*
MUD	67.4%	55.9%	
MMD	9.3%	13.2%	
TBI, %	88.4%	91.2%	*0.63*
GVHD prophylaxis, %			
CsA+MTX	44.2%	67.6%	*0.02*
TK+MTX	53.5%	30.9%	
CMV serostatus, %			
High risk (R+)	93.0%	88.2%	*0.71*
Intermediate risk (R-/D+)	4.7%	8.8%	
Low risk (R-/D-)	2.3%	2.9%	

MDS indicates myelodysplastic syndrome; CML, chronic myelogenous leukemia; ML, malignant lymphoma; ATL, adult T-cell leukemia/lymphoma; MM, multiple myeloma; SCT, stem cell transplantation; CR, complete remission; MRD, HLA-matched related donor; MUD, HLA-matched unrelated donor; MMD, HLA-mismatched donor; TBI, total body irradiation; GVHD, graft-versus-host disease; CsA, cyclosporine A; MTX, methotrexate; TK, tacrolimus ; CMV cytomegalovirus; R,recipient; D, donor.

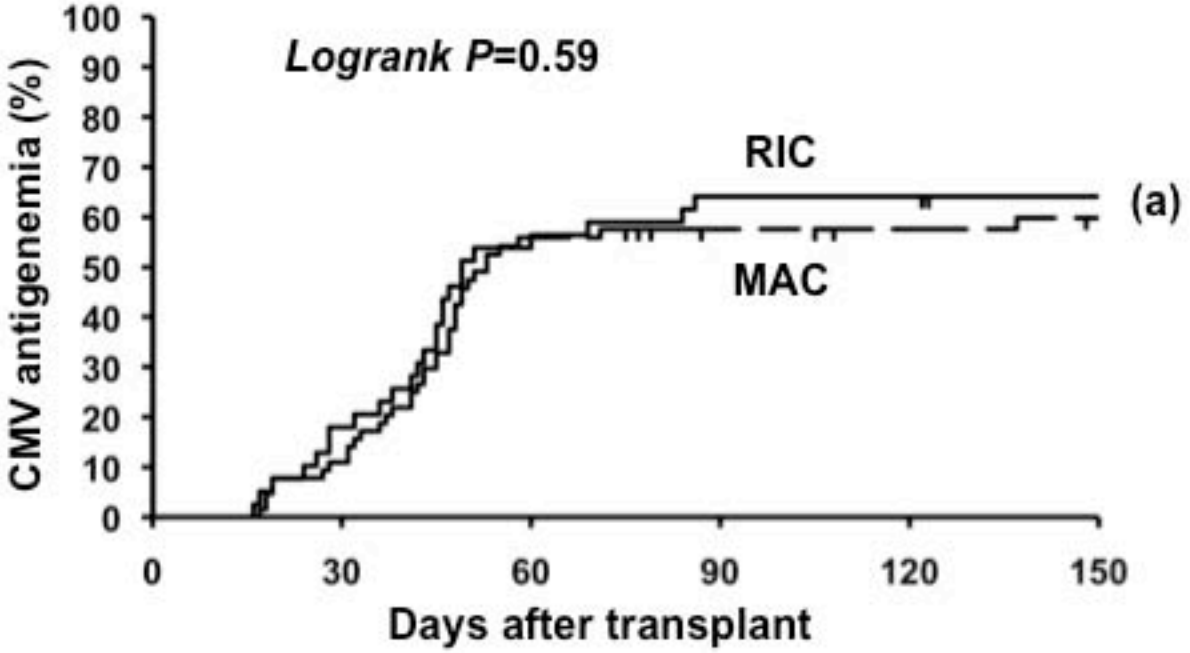
Table 2: Transplantation outocomes

		RIC	MAC	*P-value*
Grade≥3 stomatitis before engraftment		19.0%	41.5%	*0.02*
Grade≥3 diarrhea before engraftment		11.9%	34.4%	*<0.01*
Engraftment		93.0%	95.6%	*0.56*
	day, median (range)	16 (7-21)	15 (9-39)	*0.81*
Acute GVHD	overall	71.8%	78.5%	*0.44*
	onset, median (range)	33 (18-127)	20 (8-59)	*<0.01*
	grade II-IV	41.0%	52.3%	*0.26*
	grade III-IV	17.9%	15.4%	*0.73*
	gastrontestinal aGVHD	30.8%	27.7%	*0.70*
Corticosteroid		46.2%	52.3%	*0.54*
CMV Antigenemia		64.1%	57.8%	*0.59*
	onset, median (range)	day 43 (16-86)	day 43 (1-137)	
	CMV seropositive patient	69.4%	60.7%	*0.39*
	CMV seronegative patien	0.0%	25.0%	*0.62*
CMV disease	overall	5.4%	20.3%	*0.04*
	onset, median (range)	day 45,185	day 47 (25-71)	
	CMV enteritis	2.7%	12.5%	*0.20*
	CMV pneumonitis	2.7%	6.3%	*0.75*
OS (2y)		67.5%	65.0%	*0.88*

OS indicates overall survivial

Variables	CMV antigenemia	Univariate	Multivariate		CMV disease	Univariate
		P	Odds ratio (95%CI)	*P*		*P*
Conditioning regimen						
RIC	**64.1%**	***0.52***			**5.4%**	***0.04***
MAC	**57.8%**				**20.3%**	
CMV serostatus (recipient)						
Positive	**64.1%**	***0.04***	**11.8 (2.4-58.0)**	***<0.01***	**15.6%**	***0.90***
Negative	**27.2%**				**9.1%**	
Diarrhea before engraftment (grade)						
-2	**59.5%**	***0.85***			**8.3%**	***0.04***
3-	**61.5%**				**26.9%**	
Acute GVHD, overall						
Yes	**67.9%**	***<0.01***			**13.4%**	***0.74***
No	**36.0%**				**8.3%**	
Acute GVHD, grade II-IV						
Yes	**72.0%**	***0.02***			**26.5%**	***<0.01***
No	**49.1%**				**3.8%**	
Acute GVHD, gastrointestinal						
Yes	**77.4%**	***0.02***			**32.1%**	***<0.01***
No	**53.4%**				**8.2%**	
Corticosteroid						
Yes	**78.4%**	***<0.01***	**8.3 (3.0-23.2)**	***<0.01***	**24.0%**	***0.01***
No	**42.3%**				**5.9%**	

Figure 1


Logrank P=0.59
CMV antigenemia (%)
100
90
80
70
60
50
40
30
20
10
0
RIC
MAC
(a)
0
30
60
90
120
150
Days after transplant

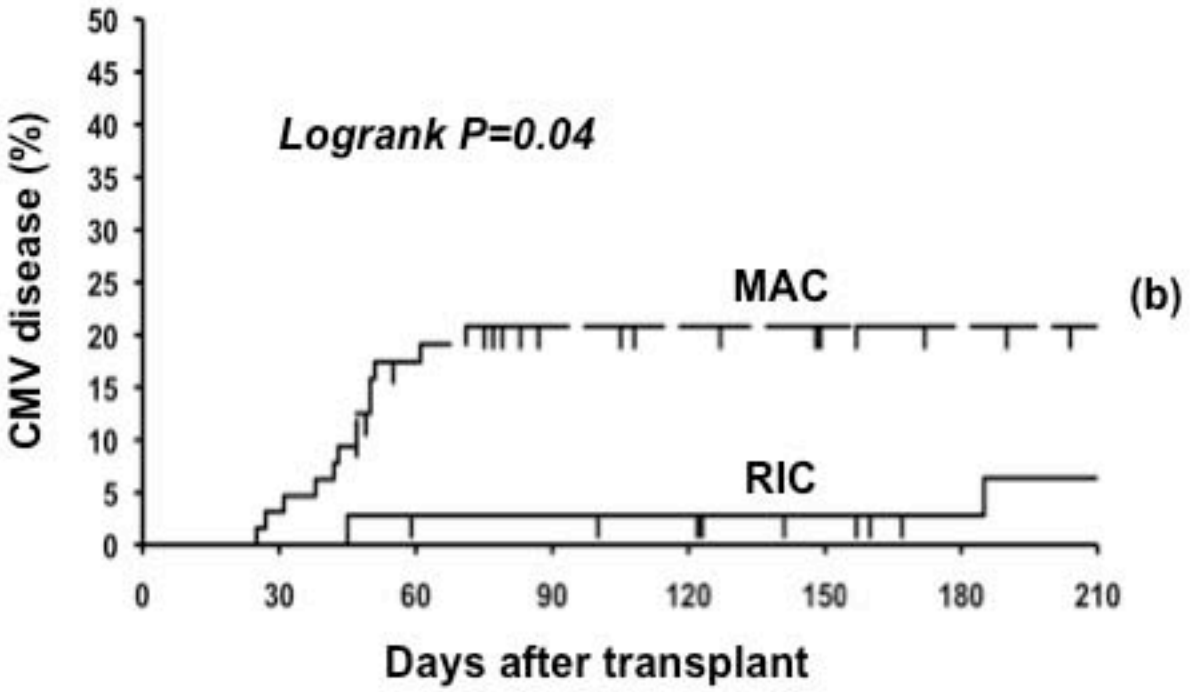
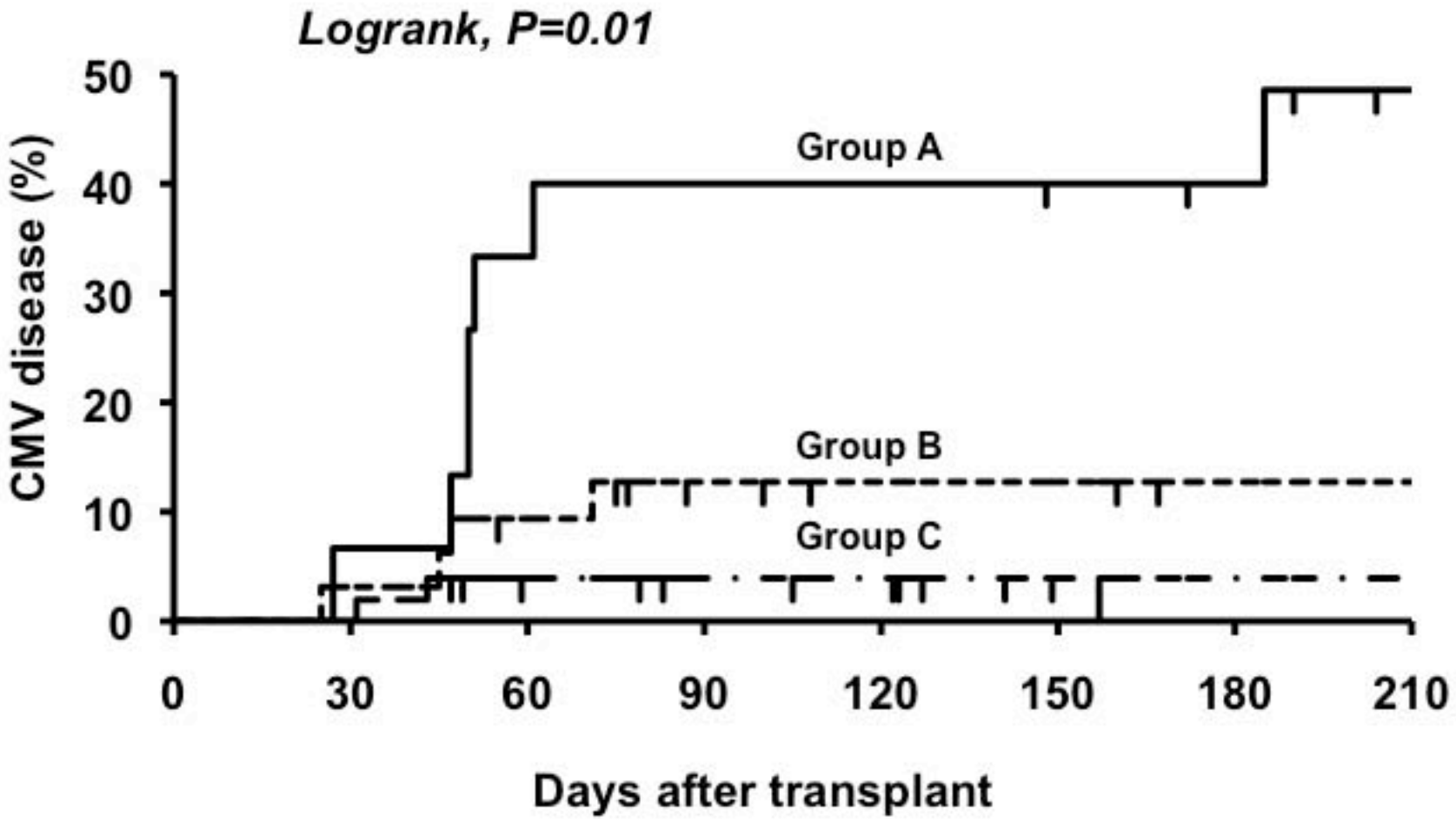

Logrank P=0.04
CMV disease (%)
50
45
40
35
30
25
20
15
10
5
0
MAC
RIC
(b)
0
30
60
90
120
150
180
210
Days after transplant

Figure 2


Logrank, P=0.01
CMV disease (%)
50
40
30
20
10
0
Group A
Group B
Group C
0
30
60
90
120
150
180
210
Days after transplant