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Severe infections after allogeneic peripheral blood stem cell transplantation: a matched-pair comparison of unmanipulated and CD34+ cell-selected transplantation

RODRIGO MARTINO, MONTSERRAT ROVIRA, ENRIC CARRERAS, CARLOS SOLANO, JORGE SIERRA, JAVIER DE LA RUBIA, MARÍA DOLORES CABALLERO, JAIME PÉREZ DE OTEYZA, JAVIER ZUAZU, JOSÉ MARÍA MORALEDA, EMILIO OJEDA, CHRISTELLE FERRÁ, DAVID SERRANO, RAFAEL DE LA CÁMARA, ALVARO URBANO-ISPÍZUA, SALUT BRUNET, FOR THE ALLOPBSCT AND INFECTIOUS/NON-INFECTIOUS COMPLICATIONS SUBCOMMITTEES OF THE GRUPO ESPAÑOL DE TRASPLANTE HEMATOPOYÉTICO (GETH)

Division of Clinical Hematology, Hospital de la Sant Creu i Sant Pau, Barcelona; Department of Hematology, Hospital Clínic i Provincial, Barcelona; Hospital Clínico Universitario, Valencia; Hospital la Fe, Valencia; Hospital Clínico Universitario de Salamanca; Hospital Ramón y Cajal, Madrid; Hospital de la Vall d'Hebron, Barcelona; Hospital Morales Meseguer, Murcia; Hospital La Paz, Madrid; Institut Catalá d'Oncología, Barcelona; Hospital Gregorio Marañón, Madrid; Hospital de la Princesa, Madrid, Spain

Correspondence: Dr. Rodrigo Martino, M.D., Servei d'Hematologia Clínica, Hospital de la Santa Creu i Sant Pau, Av. Sant Antoni M0 Claret, 167, 08025 Barcelona, Spain.
Phone: international +34.932919396. Fax: international +34.932919466. E-mail: rmartino@hsp.santpau.es

Background and Objectives. T-cell depletion of the graft delays immune recovery following allogeneic peripheral blood stem cell transplantation (PBSCT), but it is not clear whether it actually increases the risk of severe infections after the transplant.

Design and Methods. We have compared the occurrence of severe infections following 162 CD34+ cell-selected allogeneic PBSCT and 162 unmanipulated PBSCT (CD34+ and UM groups, respectively) from HLA-identical siblings.

Results. The probability of infection-related mortality (IRM) was 22% in the UM group and 31% in the CD34+ group (log-rank, p=0.2). In multivariate analyses only the use of fluconazole prophylaxis showed a protective effect on IRM in the whole set of patients, while in both transplant groups the most significant factor was the development of moderate-to-severe graft-versus-host disease (GVHD). The probability of developing cytomegalovirus (CMV) infection was 42% in the UM group and 59% in the CD34+ group (p=0.002), with no differences in CMV disease (10% and 9%, respectively). Multivariate analysis of CMV infection identified three variables associated with a higher risk in the whole set of patients: CMV positive serostatus, CD34+ transplant group and recipient age above 40 years. The development of moderate-to-severe GVHD was a significant factor only in the UM group. Disseminated varicella-zoster virus infection was more common in the CD34+ group (19% and 12%, p=0.05), as were early (< 30 days post-transplant) severe bacterial infections (28% vs 14%, p=0.002). Invasive fungal infections and pneumonias of unknown origin did not differ between groups.

Interpretation and Conclusions. Our results do not show a significant increase in the risk of dying from an opportunistic infection with CD34+-PBSCT, but the risk of CMV infection is increased, with no differences in CMV disease or mortality attributable to CMV. There is an additive effect on IRM of developing moderate-to-severe GVHD (acute or chronic) following CD34+-PBSCT, and in this subset of patients maximum efforts for the prevention and early treatment of opportunistic infections should be pursued.

Key words: peripheral blood stem cell transplantation, allogeneic, infections.

T-cell depletion (TCD) of the donor hematopoietic stem cells can successfully reduce the incidence of moderate-to-severe graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT).[1] TCD, however, increases the risk of graft failure and leukemic relapse, especially in patients with chronic myelogenous leukemia. Following HSCT all patients experience a period of profound immunodeficiency. TCD removes functionally mature T-

cells from the infused graft, and several studies have shown temporary delays in some, but not all, immune functions following TCD bone marrow transplantation (BMT) as compared to non-TCD grafts.[2-6] Similar findings have been reported following TCD allogeneic peripheral blood stem cell transplantation (PBSCT) with CD34+ cell-selection.[7,8] On the other hand, GVHD and its treatment have a profoundly negative impact on immune reconstitution following HSCT.[9] The potential benefit to immune reconstitution derived from reduction of GVHD and its therapies following TCD HSCT could be negated by the direct effect on immune recovery of TCD. The overall clinical impact of these opposing effects of TCD is still largely unknown, since there have been no large studies comparing the infectious complications after TCD and non-TCD HSCT. One of the most effective TCD methods currently in use in PBSCT is CD34+ cell-selection (CD34+-PBSCT) by immunologic methods.[10,11] CD34+-PBSCT leads to rapid hematopoietic reconstitution with an apparent reduction of acute and chronic GVHD.[10] We have compared the occurrence of severe infections following CD34+-PBSCT and unmanipulated PBSCT, with special emphasis on infection-related mortality and cytomegalovirus infections.

Design and Methods

Patient selection

Data on patients who undergo an allogeneic PBSCT in Spain are reported to the *Spanish Group for Hematopoietic Transplantation* (GETH) subcommittee on allogeneic PBSCT. Consecutive transplants are reported to separate databases for unmanipulated and CD34+ cell-selected transplants, which contain details on the underlying disease, transplantation procedure and outcome of all recipients.

This retrospective study involved adult patients who received a first allogeneic PBSCT from an HLA-identical sibling who were reported to the GETH databases. At the time the study was begun, the GETH databases included 253 unmanipulated PBSCT (UM group) and 162 CD34+-PBSCT (CD34+ group) who met these criteria. In order render baseline patient characteristics as homogeneous as possible, each patient from the CD34+ group was matched as closely as possible with a patient from the UM group with respect to age (±7 years), sex and disease phase at transplant. In the end, 130 patients per group (80%) were matched by all three variables, while 17 (10%) were matched for two and 15 (9%) for only one variable. Thus, the final groups were *matched* cohorts with 162 patients in each. The patients were transplanted in 18 centers in Spain (median 12 transplants per center, range 5-80). Of these centers, 13 performed both types of transplants, whereas in five institutions only unmanipulated transplants were done. Patients were transplanted between July 1994 and December 1998, and all surviving patients had at least nine months follow-up at the time of analysis. Each center completed a detailed questionnaire on infectious prophylaxis and the infections observed post-transplant for each patient included in the study.

Definitions

Disease phase at transplant was categorized as early (acute leukemia or poor-risk myelodysplasia in first complete remission, untreated good-risk myelodysplasia, first chronic-phase chronic myelogenous leukemia (CML), lymphoid malignancy in first remission), intermediate (acute leukemia or myelodysplasia in second or higher complete remission, accelerated-phase CML, lymphoid malignancy in second or higher remission) and advanced (refractory or relapsed acute leukemia or myelodysplasia, blastic-phase CML, refractory or relapsed lymphoid malignancy). Assessment, grading and treatment of acute and chronic GVHD were done using standard methods.[12] The infection data were collected retrospectively by each investigator until the patient's death or last follow-up. Infectious complications were defined as follows: 1) a severe bacterial infection was defined as bacteremia by any bacterial organism in a febrile patient, except for coagulase-negative staphylococci, *Micrococcus spp.* and saprophytic *Corynebacterium spp.*, which were not included in the present analysis. Bacterial infections were divided into early (occurring within the first 30 days post-transplant) and late infections (occurring beyond day 30). In patients with bacteremia, septic shock was defined by at least two of the following three criteria: systolic blood pressure < 90 mmHg (in a previously normotensive patient), heart rate > 120 per minute and respiratory rate > 28 per minute. Episodes of polymicrobial bacteremia were those in which more than one organism was isolated from one or more concurrent blood cultures, and complex polymicrobial bacteremia referred to the isolation of more than three micro-organisms; 2) invasive fungal infections were divided into candidiasis, invasive aspergillosis and other mycoses. Candidiasis was separated into candidemia (positive blood cultures for *Candida sp.* without deep organ involvement)

and visceral candidiasis (histologic and microbiological evidence of deep organ infection). Invasive aspergillosis was defined as possible (clinical signs and symptoms plus a compatible thoracic CT scan or X-ray), probable (clinical signs and symptoms, compatible X-ray findings plus a positive respiratory tract culture for *Aspergillus spp.*) and definite (positive histology for an invasive mold infection by aspergillus) infections; 3) cytomegalovirus (CMV) infection was defined as the presence of a single pp65 antigen-positive leukocyte or a positive viremia in peripheral blood, as well as documentation of CMV disease without prior positive antigenemia or viremia. CMV disease was defined as the demonstration of CMV in biopsy or autopsy specimens from clinically involved visceral sites by culture and/or histology or if CMV was detected in culture (conventional or shell-vial) of bronchoalveolar lavage (BAL) samples in the presence of new or changing pulmonary infiltrates; 4) disseminated varicella-zoster virus infection was defined as cutaneous involvement of two or more skin dermatomes or visceral involvement; 5) pneumonia of unknown origin was defined as any new radiological lung infiltrate in a febrile patient with respiratory symptoms in the absence of a known pathogen.

Patients were considered to have died from infection (ie, infection-related mortality) if death was attributable to a recent severe infection by each local investigator and/or an infection was identified at autopsy.

Outcomes

The primary outcomes that were analyzed in our study were infection-related mortality (IRM) and CMV infection. Other outcomes analyzed were bacterial infections, other severe viral infections, invasive fungal infections, pneumonia of unknown origin and other severe infections.

Statistical analysis

The chi-squared statistic or Fisher's exact test was used to establish differences in the distribution of discontinuous variables and Student's t test or Mann-Whitney's U test to compare continuous variables. All reported *p* values are two-sided, and a significance level of 0.05 was used. All infectious events were calculated from the time of transplantation using Kaplan-Meier product-limit estimates. Patients who died with an active life-threatening infection were categorized as an IRM, which was determined from the date of transplantation until death. Patients who died from other causes were censored at the date of death, and those who were still alive and progression-free at the time of reporting were censored at the last follow-up date. Since relapse or progression of the underlying malignancy may lead to immunodeficiency and secondary infections not related to the transplant procedure itself, patients were censored at the time of disease progression. Time to acute GVHD (aGVHD) grades II-IV and chronic GVHD (cGVHD) were calculated from the date of transplantation until occurrence of GVHD.

Univariate analyses of the different infections studied were performed using the log-rank test to see whether there was a difference in survival between groups, and univariate Cox regression was used to determine whether the relation was monotonous. To examine the effect of the type of transplant on the two primary outcomes of the study (IRM and CMV infection), the pre-transplant variables that had a relatively low *p* value in univariate analysis ($p < 0.5$) and those that were relevant in prior studies were used in a multivariate Cox proportional hazards regression analysis. In addition, to characterize relevant factors within each transplant type further, multivariate analyses were repeated for each group, with occurrence of aGVHD grades II-IV or extensive cGVHD included as a time-dependent covariate. The assumption of proportional hazards over time was tested for all explanatory covariates using a time-dependent covariate.

Results

Patients and transplantation procedure

Recipients of UM grafts were significantly younger than those in the CD34$^+$ group (Table 1). Underlying diseases differed somewhat, mainly due to the higher proportion of patients with acute lymphoblastic leukemia in the UM group and chronic lymphoproliferative disorders in the CD34$^+$ group. More patients in the CD34$^+$ group were in an early phase of their disease (61% CD34$^+$ group vs 44% UM group, p=0.02). There were no differences in the pretransplant donor (Do) and recipient (Re) serostatus for CMV between groups.

Conditioning regimens with total body irradiation were used in a higher proportion of recipients in the CD34$^+$ group (62% CD34$^+$ group vs 45% UM group, p=0.02). Prophylaxis for GVHD consisted in cyclosporine plus short-course methotrexate in 76% of recipients of an UM graft, while most recipients of a CD34$^+$-selected graft received cyclosporine plus prednisone (p<0.00001), as previously described.[10] As expected, the numbers of CD34$^+$ and CD3$^+$ cells infused in the graft markedly differed between groups.

Table 1. Patient characteristics (percentage in brackets).

	Unmanipulated group	*CD34⁺ group*	*p value**
Number	162	162	
Sex (M/F)	97/65	93/69	0.7
Median age [range]	39 [16-61]	39 [16-62]	0.01
Underlying disease			
Acute myelogenous leukemia	52 (32)	54 (33)	
Chronic myelogenous leukemia	28 (17)	34 (21)	
Multiple myeloma/CLL	9 (6)	25 (15)	
Acute lymphoblastic leukemia	39 (24)	18 (11)	
Non-Hodgkin's lymphoma	10 (6)	15 (9)	
Myelodysplastic syndrome	14 (9)	11 (7)	
Other	10 (6)	5 (3)	
Disease phase at transplant			0.02
Early	72 (44)	98 (61)	
Intermediate	35 (22)	24 (15)	
Advanced	55 (34)	40 (25)	
Do or Re CMV seropositive	145 (91)	139 (87)	0.3
Sex mismatch	80 (50)	74 (46)	0.5
Conditioning regimen			0.02
TBI-based	73 (45)	100 (62)	
Chemotherapy only	89 (55)	62 (38)	
Year of transplant			0.002
1994-1996	78 (48)	58 (36)	
1997-1998	84 (52)	104 (64)	
GVHD prophylaxis			<0.0001†
CyA/MTX	123 (76)	13 (8)	
CyA/PDN	37 (23)	114 (70)	
CyA only	2 (1)	32 (20)	
None	–	3 (2)	
$CD34^+$ cells ($\times 10^6$/kg) [mean, range]	6.5 [1.1-41.8]	3.8 [0.6-10.6]	<0.0001
$CD3^+$ cells ($\times 10^6$/kg) [mean, range]	332.5 [69-990]	0.36 [0.005-2.6]	<0.0001

*CMV, cytomegalovirus; Do, donor; Re, recipient. *The chi-squared test was used for categorical variables; Student's t-test or the Mann-Whitney non-parametric test was used for continuous variables. †Refers to the comparison of CyA/MTX vs CyA/PDN and CyA alone.*

Table 2. Antimicrobial prophylaxis (percentage in brackets).

	Unmanipulated group	*CD34⁺ group*	*p value**
Antibacterial prophylaxis during neuropenia			<0.001
Fluoroquinolone	140 (86)	158 (98)	
Broad-spectrum antibacterials	19 (12)	2 (1)	
Antiviral prophylaxis			0.07
Standard dose ACV until engraftment	125 (78)	113 (70)	
High-dose ACV until engraftment	25 (16)	15 (15)	
Ganciclovir after engraftment until day +100	12 (7)	24 (15)	
Antifungal prophylaxis			0.01
Fluconazole	121 (75)	109 (67)	
Amphotericin B†	10 (6)	2 (1)	
Itraconazole	16 (10)	41 (25)	
Other	15 (9)	11 (7)	
Intravenous immunoglobulin	80 (49)	124 (77)	<0.0001
G-CSF post-transplant	52 (32)	36 (22)	0.08

*ACV, acyclovir; G-CSF, granulocyte colony-stimulating factor. *The chi-squared test or Fisher's exact test was used for categorical variables. †Conventional or lipid-based formulation of amphotericin B.*

Antimicrobial prophylaxis

Table 2 shows the antimicrobial prophylaxis used in both groups of patients. All patients received antibiotic prophylaxis during neutropenia, although fluoroquinolones were used in a higher proportion of patients in the $CD34^+$ group ($p<0.001$). Most patients in both groups received antiviral prophylaxis with standard dose acyclovir during neutropenia, and ganciclovir after engraftment was given to 7% of patients in the UM group and 15% in the $CD34^+$ group. Antifungal prophylaxis also differed slightly between groups ($p=0.01$), with most patients receiving fluconazole during neutropenia. Itraconazole was used instead of fluconazole in 10% of patients in the UM group and 25% in the $CD34^+$ group. Intravenous immunoglobulin was used until day +100 post-transplant in a higher proportion of recipients of $CD34^+$ cell-selected grafts ($p<0.0001$), while the use of granulocyte colony-stimulating factor (G-CSF) did not differ between groups. All patients were housed in single rooms with either laminar airflow (5%) or HEPA filtration systems (95%) during the early post-transplant course.

Graft-versus-host disease and follow-up

The probability of developing grades II to IV aGVHD was 46% in the UM group and 18% in the $CD34^+$ group ($p<0.0001$). On day +100 similar proportions of patients were alive in both groups and thus evaluable for cGVHD. The probability of developing extensive cGVHD was 37% in the UM group and 17% in the $CD34^+$ group ($p<0.0001$). Since moderate-to-severe GVHD (both acute and chronic) is a well-known risk factor for infectious complications post-transplant, we calculated the probability of developing *either* grades II to IV aGVHD *or* extensive cGVHD, which was 64% in the UM group and 29% in the $CD34^+$ group ($p<0.0001$). This latter variable has been referred to as moder-

Table 3. Follow-up and graft-versus-host disease (probability in brackets).

	Unmanipulated group	*CD34⁺ group*	*p value**
Early deaths (< day +15)	8 (5)	4 (3)	0.2
Acute GVHD†			
Grades 0-I	84	133	
Grade II-IV	70 (46)	25 (18)	<0.0001
Day onset acute GVHD median [range]	20 [6-88]	22 [6-99]	0.4
Alive on day +100	128 (79)	136 (84)	0.3
Chronic GVHD‡			
None	51	99	
Limited	30	14	
Extensive	47 (37)	23 (17)	<0.0001
Acute GVHD II-IV or chronic extensive GVHD	64%	29%	<0.0001
Median follow-up in days [range]	447 [1-1876]	453 [8-1713]	0.6
Patients who died	119 [1-1507]	166 [8-859]	0.4
n=162	n=82	n=80	
Patients alive at last follow-up	800 [279-1876] n=162	812 [261-1713] n=82	0.6 n=80

*GVHD, graft-versus-host disease; *Probabilities were estimated with the Kaplan-Meier method, and the log-rank test was used for univariate comparisons; Student's t-test was used for continuous variables. †Excludes early deaths (unevaluable for acute GVHD). ‡Includes only patients alive on day +100.*

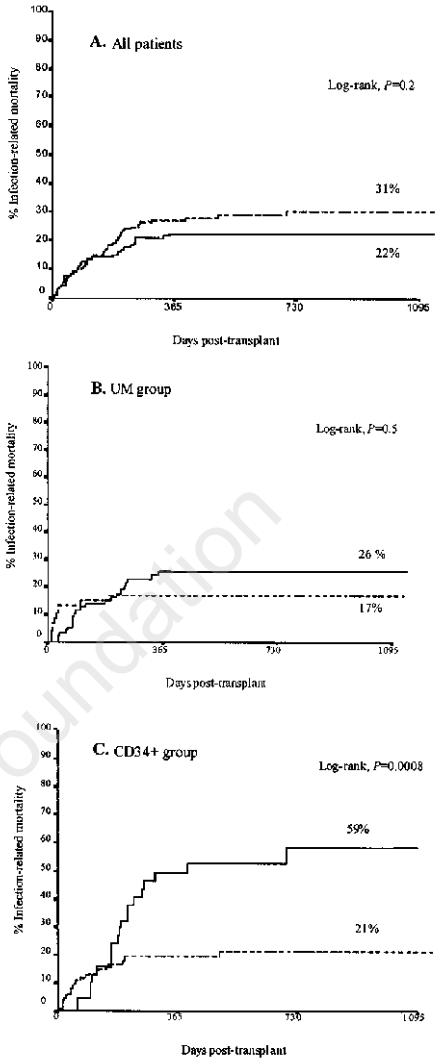

Figure 1. Probabilities of infection-related mortality after PBSC according to transplant group and development of moderate-to-severe GVHD within each group. (A) Comparison of the 162 patients who received an UM PBSCT (- - - -) and the 162 who received a CD34⁺-PBSCT (——). (B) In the UM group, comparison of the IRM in the 90 patients who developed moderate-to-severe GVHD (- - - -) with the 72 who did not (——). (C) In the CD34+ group, comparison of the IRM in the 41 patients who developed moderate-to-severe GVHD (- - - -) with the 121 who did not (——).

ate-to-severe GVHD in the text. Table 3 shows the details on GVHD and follow-up post-transplant.

The median follow-up times were similar in both groups of patients, and nearly identical proportions of patients were alive at last follow-up in the UM (49.4%) and the CD34⁺ group (50.6%).

Infection-related mortality (IRM)

Table 4 shows the causes of IRM in both groups of patients. The probabilty of IRM was 22% in the UM group and 31% in the CD34⁺ group, which was not significant in univariate analysis (p=0.2) [Figure 1a]. There were no differences in the specific causes of IRM, although bacterial infection had a borderline significance [3% in the UM group and 7% in the CD34⁺ group (p=0.08)]. Results of the Cox regression multivariate analysis of IRM are shown in Table 5. As seen, only the use of fluconazole prophylaxis during neutropenia showed a protective effect on IRM (relative risk (RR) 2.1 (95% CI 1.4-7.6), p=0.03), while the type of transplant remained non-significant (p=0.1). The subanalyses of risk factors in the two transplant groups are also shown in Table 5. In the CD34⁺ group, the multivariate analysis found three variables associated with a higher risk of IRM: non-use of fluconazole [RR 3.7 (95% CI 1.4-9.9), p=0.01], the use of G-CSF post-transplant [RR 1.5 (95% CI 1.1-2.2), p=0.04] and the development of moderate-to-severe GVHD

Table 4. Overall infectious mortality and causes of death (probability in parentheses).

	Unmanipulated group	*CD34⁺ group*	*p value**
Infection-related mortality	32 (22)†	44 (31)‡	0.2
Causes of death from infection			
Bacterial infection	5 (3)	12 (7)	0.08
Pneumonia of unknown etiology	15 (9)	14 (9)	0.9
CMV disease	4 (3)	8 (6)	0.3
Other viral infections	1	1	
Invasive fungal infections	9 (7)	12 (9)	0.6
Invasive aspergillosis	8	9	
Mucormycosis	1	1	
Fusariosis	-	1	
Disseminated candidiasis	-	1	
Toxoplasmosis	-	1	
Progressive mulifocal leukoencephalopathy	-	1	

**Probabilities were estimated with the Kaplan-Meier method, and the log-rank test was used for univariate comparisons. †Two patients had polymicrobial infections. ‡Four patients had polymicrobial infections.*

[RR 6.1 (95% CI 3.1-12), $p<0.0001$]. In the UM group, the multivariate analysis found three variables associated with a higher risk of IRM: steroid prophylaxis post-transplant [RR 2.4 (95% CI 1.1-5.3), $p=0.03$], the use of G-CSF post-transplant [RR 2.4 (95% CI 1.1-5.6), $p=0.05$] and the development of moderate-to-severe GVHD [RR 3 (95% CI 1.3-6.7), $p=0.008$]. Figures 1b and 1c show the Kaplan-Meier curves of IRM according to the development of moderate-to-severe GVHD in both groups of transplants. As seen in Figure 1c, the difference in IRM according to GVHD was especially relevant in the CD34⁺ group, in which the probability of IRM was 59% in the 41 patients who developed GVHD vs 21% in the 121 subjects who did not develop GVHD ($p=0.0008$).

Viral infections

Table 6 details the life-threatening viral infections observed in both groups. The probability of developing CMV infection was 42% in the UM group and 59% in the CD34⁺ group ($p=0.002$) [Figure 2a]. The probability of CMV disease did not, however, differ between groups (10% and 9%, respectively). Treatment for CMV infection based on a positive antigenemia or viremia did not differ between groups, and consisted in ganciclovir in 94% and 87% of cases, and foscarnet in 4% and 13%, respectively ($p=0.1$). One patient in each group was not treated for CMV disease, while all other cases were treated with either ganciclovir alone (n=9) or ganciclovir combined with high-dose intravenous immunoglobulin (n=15). Four patients (3%) in the UM group and eight (6%) in the CD34⁺ group died from CMV disease ($p=0.3$). Results of the Cox regression multivariate analysis of CMV infection are shown in Table 7. The three variables associated with a higher risk were: transplant group [RR 1.5 (95% CI 1-2.3), $p=0.03$], CMV serostatus [RR 7.7 (95% CI 2.5-24.5), $p=0.0005$] and recipient age [RR 1.5 (95% CI 1.1-2.1), $p=0.03$]. The subanalyses of risk factors in the two transplant groups are also shown in Table 7. In the

Table 5. Summary of the results of multivariate analyses for infection-related mortality.

	Overall analysis		*CD34+ group*		*UM group*	
	Mutivariate p	*RR (95% CI)*	*Mutivariate p*	*RR (95% CI)*	*Mutivariate p*	*RR (95% CI)*
Transplant group (CD34⁺ vs unmanipulated)	0.1			–		–
Recipient age (> 40 vs ≤ 40)	0.9		0.9		0.6	
Disease phase (early vs intermediate/advanced)	0.1		0.2		0.8	
TBI in conditioning (no vs yes)	0.4		0.3		0.5	
CMV serostatus (positive* vs negative)	0.3		0.1		0.5	
Year of transplant (1997-1998 vs 1994-1996)	0.08		0.1		0.08	
Fluoroquinolone prophylaxis (no vs yes)	0.2		NI		NI	
Standard-dose acyclovir prophylaxis (no vs yes)	0.7		NI		NI	
Fluconazole prophylaxis (no vs yes)	0.03	2.1 (1.4-7.6)	0.01	3.7 (1.4-9.9)	0.5	
IVIG post-transplant (no vs yes)	0.3		0.1		0.5	
G-CSF post-transplant (yes vs no)	0.06		0.04	1.5 (1.1-2.2)	0.05	2.4 (1.1-5.6)
CD34⁺ cell dose (below vs above the median per group)	NI		0.5		0.3	
Steroid prophylaxis (yes vs no)	NI		0.7		0.03	2.4 (1.1-5.3)
Grade II-IV aGVHD or extensive cGVHD (yes vs no)	NI		<0.0001	6.1 (3.1-12)	0.008	3 (1.3-6.7)

*NI, not included in the model. *Positive pretransplant IgG for CMV in the donor or recipient.*

Table 6. Life-threatening viral infections (probability in brackets).

	Unmanipulated group	*CD34⁺ group*	*p value**
Cytomegalovirus			
Cytomegalovirus infection	57 (42)†	87 (59)‡	0.002
Median day onset [range]	52 [7-399]	44 [10-504]	0.4
Cytomegalovirus disease	14 (10)	12 (9)	0.6
Median day onset, range	58 [20-160]	50 [30-256]	0.5
Site of disease			
Pneumonia	8	9	
Gastrointestinal tract	4	3	
Other	2§	-	
Other viral infections	16 (19)	22 (22)	0.4
Disseminated VZV infection	8 (12)	18 (19)	0.05
Herpes simplex type 1°	3	1	
Adenovirus#	1	1	
RSV pneumonia	-	1	
Influenzae A pneumonia	-	1	
Polyomavirus-associated cystitis	4	-	

*RSV, respiratory syncytial virus; VZV, varicella-zoster virus. *Probabilities were estimated with the Kaplan-Meier method, and the log-rank test was used for univariate comparisons; Mann-Whitney's non-parametric test was used for continuous variables. †Five patients developed CMV disease without prior documentation of a positive antigenemia or viremia. The other 52 cases developed a positive CMV antigenemia or viremia as the first evidence of infection. ‡Five patients developed CMV disease without prior documentation of a positive antigenemia or viremia. The other 82 cases developed a positive CMV antigenemia or viremia as the first evidence of infection. §Includes one case of hepatitis and one disseminated infection. °Includes three cases of esophagitis and one pneumonia. #Includes one case of colitis and one disseminated infection.*

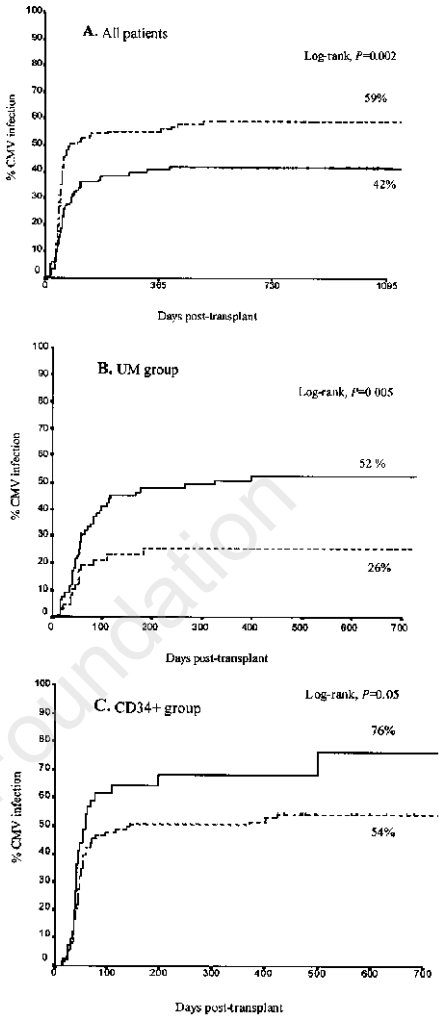

Figure 2. Probabilities of developing cytomegalovirus (CMV) infection after PBSC according to transplant group and development of moderate-to-severe GVHD within each group. (A) Comparison of the 162 patients who received an UM PBSCT (- - - -) and the 162 who received a CD34⁺-PBSCT (——). (B) In the UM group, comparison of CMV infection in the 90 patients who developed moderate-to-severe GVHD (- - - -) with the 72 who did not (——). (C) In the CD34⁺ group, comparison of CMV infection in the 41 patients who developed moderate-to-severe GVHD (- - - -) with the 121 who did not (——).

CD34⁺ group, the multivariate analysis found two variables associated with a higher risk of CMV infection: CMV serostatus [RR 8.2 (95% CI 2-33.8), p=0.004] and the dose of CD34⁺ cells/kg recipient weight infused in the graft [RR 1.6 (95% CI 1-2.9), p=0.05]. In the UM group, the multivariate analysis found four variables associated with a higher risk of infection: CMV serostatus [RR 7 (95% CI 1.1-51.9), p=0.04], recipient age [RR 1.8 (95% CI 1.1-3.2), p=0.04], the dose of CD34⁺ cells/kg recipient weight infused in the graft [RR 1.5 (95% CI 1.1-2.8), p=0.05] and the development of moderate-to-severe GVHD [RR 2.7 (95% CI 1.5-4.7), p=0.0004]. Figures 2b and 2c show the Kaplan-Meier curves of CMV infection according to the development of moderate-to-severe GVHD in both groups of transplants. As seen in Figure 2b, the difference in CMV infection according to GVHD is especially relevant in the UM group, with probabilities of 52% in the 90 patients who developed GVHD vs 26% in the 72 subjects who did not develop GVHD (p=0.005). In the CD34⁺ group, however, the probabilities were high in both subgroups although higher in those who developed GVHD (76% and 54%, respectively, p=0.05; Figure 2c). With respect to CMV disease, 12/14 patients in the UM group and 9/12 in the CD34⁺ group had previously developed moderate-to-severe GVHD. Other life-threatening viral infections are shown in Table 6. The probability of disseminated varicella-zoster virus infection was higher in the CD34⁺ group (19%) than in the UM group (12%) [p=0.05]. Other viral infections were uncommon, and there

Table 7. Summary of the results of multivariate analyses for cytomegalovirus infection.

	Overall analysis		*CD34⁺ group*		*UM group*	
	Mutivariate p	*RR (95% CI)*	*Mutivariate p*	*RR (95% CI)*	*Mutivariate p*	*RR (95% CI)*
Transplant group (CD34⁺ vs unmanipulated)	0.03	1.5 (1-2.3)	-		-	
Recipient age (> 40 vs ≤40)	0.03	1.5 (1-2.1)	0.2		0.04	1.8 (1-3.2)
Disease phase (early vs intermediate/advanced)	0.8		0.3		0.6	
TBI in conditioning (no vs yes)	0.1		0.2		0.2	
CMV serostatus (positive* vs negative)	0.0005	7.7 (2.5-24.5)	0.004	8.2 (2-33.8)	0.04	7 (1.1-51.9)
Year of transplant (1997-1998 vs 1994-1996)	0.1		0.7		0.2	
Standard-dose acyclovir prophylaxis (no vs yes)	0.3		0.06		0.5	
IVIG post-transplant (no vs yes)	0.6		0.7		0.2	
CD34+ cell dose (below vs above the median per group)	NI		0.05	1.6 (1-2.9)	0.05	1.5 (1.1-2.8)
Grade II-IV aGVHD or extensive cGVHD (yes vs no)	NI		0.2		0.0004	2.7 (1.5-4.7)

*NI, not included in the model. *Positive pretransplant IgG for CMV in the donor or recipient.*

were no cases of post-transplant lymphoproliferative disease.

Bacterial infections and pneumonia

Early invasive bacterial infections occurred in 28% of patients in the CD34⁺ group and 14% in the UM group (p=0.002). Table 8 shows the details of bacterial infections. Late infections were also somewhat more frequent in the CD34⁺ group, although the differences were not statistically significant. Five patients (3%) in the UM group and 12 (7%) in the CD34⁺ group died from a bacterial infection (p=0.08). Since the use of steroids as GVHD prophylaxis was unevenly distributed between transplant groups and these drugs may predispose to bacterial infections, we analyzed the probability of early and late infections in both groups according to the use or not of steroids as prophylaxis. In both groups the probabilities of both early and late infections did not differ with the use of steroids (data not shown).

A large number of species were isolated in both early and late infections, without identifiable differences between transplant groups (see Table 8 for details). Viridans group streptococci were the most frequent group of bacteria isolated in early infections (n=26 cases), followed by enterobacteria (n=23), while *Pseudomonas aeruginosa* (n=3) and other non-glucose fermenting Gram-negative bacilli (n=6) were less frequently involved. In late bacterial infections, however, *Pseudomonas aeruginosa* (n=7) and other non-glucose fermenting Gram-negative bacilli (n=17) were the most frequent isolates, followed by enterobacteria (n=11), Gram-positive cocci (n=12) and other Gram-neg-

Table 8. Severe bacterial infections and idiopathic pneumonias after transplant (probability in parentheses).

	Unmanipulated group	*CD34⁺ group*	*p value**
Early infections (≤ day +30)	23 (14)†	46 (28)‡	0.002
Bacteremia	19	31	
Septic shock	3	8	
Pneumonia	-	2	
Other	1	5	
Late infections (after day +30)	27 (17)§	34 (21)°	0.3
Bacteremia	11	11	
Catheter-related infection	6	9	
Septic shock	3	6	
Pneumonia	4	5	
Other	3	3	
Idiopathic pneumonia	28 (24)	25 (20)	0.6

**Probabilities were estimated with the Kaplan-Meier method, and the log-rank test was used for univariate comparisons. †The species isolated included viridans group streptococci (n=9), Escherichia coli (n=5), Pseudomonas aeruginosa (n=3), Staphylococcus aureus, Stenotrophomonas maltophilia (n=2 each), Enterococcus faecalis and Acinetobacter sp. (n=1 each); one infection was polymicrobial. ‡The species isolated included viridans group streptococci (n=17), Escherichia coli (n=15), Pseudomonas aeruginosa (n=3), Streptococcus pneumoniae, Enterococcus sp., Fusobacterium nucleatum (n=2 each) and Acinetobacter sp., Staphylococcus aureus, Citrobacter freundii, Stenotrophomonas maltophilia, Burkholderia cepacia, Klebsiella pneumoniae, Capnocytophaga sputigena and Proteus mirabilis (n=1 each); three infections were polymicrobial.*
§The species isolated included Pseudomonas aeruginosa (n=5), Escherichia coli (n=4), Stenotrophomonas maltophilia, Campylobacter jejuni, Enterococcus faecalis (n=2 each), Listeria monocytogenes, Mycobacterium tuberculosis, Pseudomonas fluorescens, Serratia marcenses, Staphylococcus aureus, Salmonella enteritidis, Proteus mirabilis, Ochromobacter anthropi, Bordetella bronchiseptica (n=1 each) and complex polymicrobial bacteremias (n=2).
°The species isolated included Streptococcus pneumoniae (n=3), Pseudomonas fluorescens, Pseudomonas aeruginosa, Escherichia coli, Listeria monocytogenes (n=2 each), Campylobacter jejuni, Aeromonas caviae, Streptococcus pyogenes, Stenotrophomonas maltophilia, Staphylococcus aureus, Streptococcus thermophilus, Enterococcus faecalis, Enterobacter cloacae, viridans group streptococci, Klebsiella pneumoniae, Pseudomonas putida, Nocardia asteroides, Serratia marcenses, Mycobacterium tuberculosis, Salmonella enteritidis (n=1 each), non-speciated non-glucose fermenting Gram negative rods (n=3) and complex polymicrobial bacteremias (n=3).

ative bacteria (n=5), with five cases of complex polymicrobial infections.

The probability of developing pneumonia of unknown origin was similar in both groups (24% in the UM group and 20% in the CD34+ group [p=0.6]), as was death from this cause (9% in both groups).

Invasive fungal infections

The probability of developing an invasive fungal infection was the same in both transplant groups (20 cases [15%] in the UM group and 21 cases [15%] in the CD34+ group), as was the probability of death from this cause (7% in the UM group and 9% in the CD34+ group [p=0.6]). In the UM group there were six cases of possible, four cases of probable and four cases of definite invasive aspergillosis. In the CD34+ group, there were three, one and eleven cases of each. There were three and four cases of candidemia and one and two cases of invasive visceral candidiasis in each group, respectively. Other invasive mycoses included one penicillinosis and one mucormycosis in the UM group, and one mucormycosis, one disseminated cryptococcosis and one fusariosis in the CD34+ group. The median times to onset of non-candida fungal infections was 48 days (range 4-522) in the UM group and 99 days (range 6-304) in the CD34+ group. Moderate-to-severe GVHD preceded the onset of these infections in 14/16 and 11/18 cases, respectively. Causes of death from fungal infection are detailed in Table 4.

Other life-threatening infections

There were three cases of encephalitis of unknown origin, two in the UM group and one in the CD34+ group, none of which was fatal. There was one case of *Pneumocystis carinii* pneumonia in each group, both of which resolved with appropriate therapy. One patient in the CD34+ group died from toxoplasmosis on day +63 and another died from progressive multifocal leukoencephalopathy on day +390.

Discussion

Immunologic reconstitution following TCD allogeneic HSCT (both BMT and PBSCT) has been studied in detail.[2-9] Although quantitative and qualitative differences in T-cell reconstitution among TCD and non-TCD transplants are frequent during the first months post-transplant, the impact of such differences on the occurrence of severe infections has not been studied in detail. Although life-threatening opportunistic infections are an important cause of transplant-related mortality, it is uncertain whether TCD modifies the risk of dying from an opportunistic infection. Results of the current study indicate that CD34+-PBSCT is not associated with a statistically significant higher risk of dying from an opportunistic infection, although a trend is seen in univariate analysis. When analyzing the risk factors for IRM in each transplant type, moderate-to-severe GVHD was the main risk factor for IRM, a logical finding since GVHD and its treatment confer a state of severe immune deficiency that predisposes to all types of opportunistic infections.[13-17] The additive effect of CD34+-PBSCT and GVHD on the IRM was particularly impressive (59% probability of IRM in the 41 recipients of a CD34+-PBSCT who developed moderate-to-severe GVHD). In an early study from the Memorial Sloan Kettering Cancer Center BMT team, the infectious complications observed in 48 recipients of TCD BMT were compared to those in 24 non-TCD transplant recipients.[2] There were no significant differences between groups, although trends were observed in the rates of early bacterial sepsis (16/48 vs 5/24, p=0.08) and varicella-zoster virus infections (5/48 vs 0/24, p=0.1), as seen in our study. Later studies from this same group focusing only on TCD BMT recipients determined that immune reconstitution is delayed and life-threatening infections are increased in older adults and in patients receiving antithymocyte globulin and high-dose steroids as graft rejection prophylaxis.[5,6] In these studies the probability of developing a life-threatening infection in 142 adults was 25%. In an early study from Germany which compared the infections seen in 40 recipients of TCD BMT with 27 recipients of non-TCD grafts, early bacterial infections were more common in the non-TCD group (70% vs 43%, p=0.02), and the IRM was also higher in this patient group (43% vs 19%, p=0.05).[18] It is unlikely, however, that these results are applicable nowadays due to signficant advances in supportive care and transplant techniques.

Two studies have recently compared immune recovery and infectious complications following CD34+-PBSCT and UM-PBSCT.[7,8] The small sample sizes (12 to 18 patients per group) do not allow adequate analyses, although in one study the probability of CMV antigenemia was higher in the CD34+ group (67% in the CD34+ group vs 33% in the UM group, p=0.04).[8] A study of CMV infection following allogeneic PBSCT has recently been reported in abstract form by the *EBMT Infectious Diseases Working Party*.[19] This study included children and adults and both related and unrelated donor transplants. The frequency of CMV infection was 32% among 116 TCD PBSCT and 25% among

352 UM transplants, and the frequency of CMV disease was 13% and 5%, respectively. CMV-related mortality was also higher in the TCD group (8% vs 1.7%, respectively, p=0.01), and the mortality among patients with CMV disease was 8/15 (53%) and 6/18 (33%), respectively. In our study the risk of developing CMV infection was higher in the CD34+ group in univariate and multivariate analyses, together with other variables such as older age and CMV seropositive status. When analyzing the risk factors for CMV infection in each transplant type, moderate-to-severe GVHD was a significant risk factor only in the UM group, while the CMV serostatus and higher infused doses of CD34+ cells were significant in both groups. TCD transplantation is a known risk factor for CMV infection,[15,17,20,21] and this is due to the elimination of CMV-reactive specific mature T-cell clones from the graft which confer immunity to the virus during the first months post-transplant.[17,22] GVHD is also an important risk factor for CMV infection and disease,[13-15,17] and in our study the highest probability of infection was seen in recipients of CD34+-PBSCT who developed moderate-to-severe GVHD. The independent role of TCD in increasing the risk of CMV infection is highlighted by the high incidence of both with CD34+ cell-selected PBSCT in the autologous setting, in which CMV has traditionally been a less dreaded menace.[23-25] The protective effect of higher infused doses of CD34+ cells on CMV infection has not to our knowledge been previouly reported, although high stem cell doses have been reported to reduce the transplant-related mortality and improve other transplant outcomes following related BMT, unrelated BMT, related PBSCT and syngeneic HSCT.[26-30]

The risk of early life-threatening bacterial infections in the CD34+ group was double that in the UM group. It is unlikely that the exclusion of infections by coagulase-negative staphylococci and other skin colonizers from our analysis or the slight differences in baseline patient characteristics are responsible for these differences. The use of prophylactic steroids, however, has been shown to increase the risk of early blood-stream infections in one randomized study,[31] although a later study did not show any statistically significant difference.[32] Since a much larger proportion of patients in the CD34+ group received prophylactic steroids, we analyzed, in both transplant groups, the probability of developing an early bacterial infection in patients who received prophylactic steroids and those who did not receive steroids and found no differences. On the other hand, late bacterial infections did not differ between groups. Of note the large proportion of late infections caused by non-glucose fermenting Gram-negative bacilli, a heterogeneous group of opportunistic teluric bacteria which are an increasing cause of infections in patients with hematologic malignancies.[33-35]

In summary, the results from our study suggest that TCD of allogeneic PBSCT with CD34+ cell-selection does not significantly increase the risk of dying from an opportunistic infection. The risk of CMV infection is increased with CD34+-PBSCT, although there were no differences in CMV disease or mortality attributable to CMV. Of special relevance, however, is the additive effect on IRM of developing moderate-to-severe GVHD (acute or chronic) following a CD34+-PBSCT,[36] and in this subset of patients maximum efforts for the prevention and early treatment of opportunistic infections should be pursued.

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RM and MR designed the study and were responsible for data management, statistical analysis and preparation and submission of the various versions of the manuscript. SB and AUI made an important contribution to data management. All other co-authors (EC, CS, JS, JDR, MDC, JPO, EO, JZ, JMM, CF, DS and RDC) had a relevant participation in patients' recruitment (> 5% of patients included in the study) and revised the different versions of the manuscript.

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Disclosures

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Potential implications for clinical practice

CD34+ cell-selected PBSCT increases the risk of suffering from several severe infections after CD34+ cell-selected PBSCT. CD34+ cell-selected PBSCT recipients who develop GVHD are at a very high risk of developing severe infections and dying from an infection, justifying the use of intense infectious prophylaxis in this situation.

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