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ORIGINAL ARTICLE

Lymphocyte subsets recovery following allogeneic bone marrow transplantation (BMT): CD4+ cell count and transplant-related mortality

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To assess the kinetics of lymphocyte subset recovery, 758 allografted patients were monitored by surface markers (CD3, CD4, CD8, CD56), with a 5-year follow-up. The donor was a matched sibling donor (MSD) ($n = 502$) or an alternative donor (family mismatched or unrelated, AD) ($n = 256$). The stem cell source was bone marrow for all patients. CD4+ cell recovery was influenced—in univariate analysis—by three factors: donor type, patient age and GvHD. This was not the case for CD8+ and CD56+ cells. The median CD4+ cell count on day +35 after HSCT was 86/μl. Patients achieving this CD4+ cell count had significantly lower transplant-related mortality (TRM) compared to patients who did not achieve this CD4+ cell count (20 vs 39%, $P = 0.00001$), due to a lower risk of lethal infections (24 vs 47%, $P = 0.0003$). In multivariate analysis MSD (RR 3.45, $P = 0.0001$) and recipient age less than 16 years (RR 3.23, $P = 0.003$) were significantly associated with a better CD4+ cell recovery. CD4+ counts on day +35 was predicted TRM (RR = 1.97, $P = 0.0017$) together with acute GvHD grade II–IV (RR 1.59, $P = 0.0097$). No difference of TRM was observed for CD8+ and CD56+ cell counts.

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Introduction

Despite successful homing and engraftment of stem cells into host haemopoietic tissue, donor-derived immune reconstitution in the transplant recipient may not readily achieve functional maturation for months or years, if at all, after haemopoietic stem cell transplantation (HSCT). HSCT using either a closely matched unrelated donor or a partially mismatched family member has been proposed as an alternative strategy for patients lacking a matched sibling donor (MSD).[1,2] An intensive conditioning regimen to facilitate the engraftment and an increased immunosuppressive regimen for GvHD prophylaxis have been used in these high risk procedures. Thus, due to the profound immunosuppression state and prolonged neutropenia, patients transplanted from alternative donors (AD) have been shown to have a higher rate of infectious complications. Immune reconstitution is an important component of successful allogeneic HSCT, perhaps the most relevant, since viral, fungal and bacterial infections contribute significantly to morbidity and mortality.[3,4]

Several studies have analyzed immune reconstitution after allogeneic stem cell transplantation. The number of NK cells, as monitored by CD56 or CD16 expression on peripheral blood lymphocytes, rises rapidly after transplant,[5–7] and returns quickly to the normal range while CD8+ cells frequently remain higher for a longer lasting period after transplant.[8] CD4+ cells, especially those expressing CD45RA antigen (naivety marker), are depressed for a long time after transplant, while the recovery of CD4+ expressing CD45RO or CD29 antigen (memory/effector cells) is quicker.[9] The imbalance of T-cell subsets with an inversion of the helper/suppressor cells still needs to be fully understood, despite having been described many years ago.[10] It may be associated with acute GvHD (aGvHD) and chronic GvHD (cGvHD) as both have a negative impact on immune reconstitution.[11,12] Moreover, the age of recipients affects both CD4+ recovery and response to mitogens.[13–17] It has been suggested that reconstitution and maintenance of effective T-cell immunity after HSCT is dependent on education of T-cell precursors in the thymus.[18–20] These observations prompted research on factors affecting the reconstitution of T-cell immunity through the thymic dependent pathway. By the use of phenotypic markers of T-cell naivety (primarily CD45+RA antigen), initial studies showed that increasing patient age has an adverse effect on the regeneration of naive CD4+ T cells, probably due to age-related thymic involution. These observations were confirmed by TCR rearrangement excision circlet (TREC) assay to measure thymic output.[21,22]

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If some factors such as GvHD and patient age have an important role on immune reconstitution and if immune reconstitution has a decisive role on successful HSCT, then the monitoring of immune recovery may be useful to assess the risk of transplant-related mortality (TRM).

Our study shows lymphocyte recovery in a large number of allograft recipients, factors affecting CD4+ lymphocyte recovery and the impact of recovery on TRM.

Patients and methods

Objective of the study

The first priority of our study was to gain an overall picture of early and late CD3+CD4+, CD3+CD8+ and CD3−CD56+CD16+ cell recovery following HSCT and bone marrow (BM) as stem cell source only. Second, we carried out a univariate and multivariate analysis to analyze factors associated with the early CD4+ cell recovery. Then, we carried out cumulative incidence (CI) curves of TRM as end point according to CD4+, CD8+, CD3−CD56+CD16+ median cell count on day 35. Finally a multivariate analysis for TRM as end point was performed to analyze factors associated with higher TRM risk.

Patients' characteristics

Seven hundred fifty-eight patients underwent allogeneic BM transplantation (BMT) at our unit (Table 1 for clinical details) from January 1990 to August 2001. The conditioning regimen was cyclophosphamide (60 mg/kg once daily i.v.) on each of two consecutive days (total dose 120 mg/kg) followed by total body irradiation 9.9–12 Gy for acute leukemia and chronic myeloid leukemia (CML); cyclophosphamide 50 mg/kg once daily i.v. on each of four consecutive days (total dose 200 mg/kg) for severe aplastic anemia; the combination of Thiotepa (10 mg/kg i.v. on day 1) with cyclophosphamide (50 mg/kg once daily i.v. on day 2 & 3) (reduced intensity regimens) was used mainly for patients with myelodysplastic syndromes, non-Hodgkin's lymphoma or older patients (>45 years of age). MSD recipients received GvHD prophylaxis consisting in CYA (1–2 mg/kg from day −1) and methotrexate 15 mg/m^2 on day +1, and 10 mg/m^2 on day +3 and +6 or CYA alone (1–2 mg/kg from day −1). AD recipients received CYA (1–2 mg/kg from day −1), methotrexate 15 mg/m^2 on day +1 and 10 mg/m^2 on day +3 and +6 and *in vivo* T-cell depletion with anti-thymocyte globulin (Thymoglobulin, Genzyme, Cambridge, MA, USA), 3.5 mg/kg/day on day −4, −3, −2. Transplants were performed in rooms with positive pressure filtered flow. Antifungal prophylaxis of oral nystatin and then mepartricin (an absorbable polyene) was used until the advent of fluconazole (now the standard fungal prophylaxis), until day +75. Secondary prophylaxis with amphotericin or voriconazole was given if the patient was transplanted with a known history of *Aspergillus* infection or other fluconazole resistant fungi. Antiviruses: MSD patients received acyclovir, while the AD group received ganciclovir or foscarnet as CMV prophylaxis (according to the clinical protocol used) was given to all patients. Gut decontamination was achieved with quinolones. We used ciprofloxacin orally unless the patient became febrile. At that point the patient was treated with intravenous antibiotics, the first choice was an aminoglycoside and a cephalosporin and vancomycin was added after 3 days if the patient had no fever regression. Total parenteral nutrition was administrated if stomatitis developed. BM was the source of stem cells for all patients.

The local ethical committee at the San Martino Hospital approved the study. Informed consent was obtained from the patients or their parents.

Table 1 Clinical data of patients undergoing allogeneic stem cell transplants

Donor		
	MSD	*AD*
	$N=502$	$N=256$
Gender M/F	271/231	129/127
Age (median/range)	32 (4–67)	34 (8–72)
Recipient		
Gender M/F	291/211	164/92
Age (median/range)	33 (4–65)	34 (7–56)
<16	29 (6%)	2 (1%)
⩾16	473 (94%)	254 (99%)
Abo compatibility		
Identical	71.3%	53.3%
Minor incompatibility	15.3%	16.1%
Major incompatibility	13.4%	30.6%
Disease		
SAA	45 (9%)	4 (2%)
Acute leukemia	215 (43%)	69 (27%)
Chronic myeloid leukemia	170 (34%)	125 (49%)
MDS	14 (3%)	17 (7%)
Multiple myeloma	12 (2%)	2 (1%)
Non-Hodgkin's lymphoma	10 (2%)	18 (7%)
Sec acute leukemia	9 (2%)	6 (2%)
Other	27 (5%)	23 (6%)
Conditioning regimen TBI y/n	386/116	191/65
Disease phase		
Early/advanced	332/170	105/151
Interval diagnosis/transplant	267.5 (29–6240)	894 (84–5081)
GvHD prophylaxis		
CYA	34%	2%
CYA+MTX	65%	95%
No prophylaxis	1%	2%
Cells		
Cells per kg	4.2×10^8 (0.8–28)	3.57×10^8 (1–20)

Abbreviations: MDS = myelodysplasia; SAA = severe aplastic anaemia. Early disease is first complete remission for acute leukemia patients, while for chronic myeloid leukemia it was the 1st chronic phase with diagnosis within 1 year. All other patients were considered as advanced.

Cell preparation

Heparinized blood samples were obtained from patients at various time intervals after HSCT: day 20 (±5), day 35 (±7), day 75 (±10), day 150 (±10), day 365 (±30), day 730 (±30) and day 1825 (±60). Freshly isolated samples were analyzed without any other manipulation.

Immunophenotyping of lymphocyte subsets

Lymphocyte subset analysis was carried out with a workstation Coulter Q-Prep. Cell antigen analysis was carried out using a direct immunofluorescent technique, using a panel of conjugated FITC/PE monoclonal antibodies. CytoStat Coulter CD3/CD4, CD3/CD8 for T cells and CD3/CD56/16 for NK cells. Briefly, $0.5–1 \times 10^6$ cells were incubated with CD3/CD4, CD3/CD8, CD3/CD56−CD16 monoclonal antibodies for 30 min at 4 °C in the dark. Then, red cells were lysed with ammonium chloride obtained from Ortho for 15 min in the dark. Cells were washed twice and maintained at 4 °C until analysis. Fluorescence was analyzed with Coulter Epics Profile II. Isotypically matched mouse immunoglobulin conjugated FITC or PE was used as a negative control in all tests. Immunological recovery was expressed as an absolute number ($\times 10^9$/l) and the median values were compared, minimum and maximum value per group are also reported.

No functional tests were performed.

Definitions

The day of stem cell infusion was defined as day 0. Neutrophil and platelet engraftment was defined as the first of three consecutive days with neutrophil count greater than 0.5×10^9/l and platelet count greater than 50×10^9/l, respectively. Patients with evidence of donor engraftment who survived more than 14 days and more than 100 days were evaluated for the development of acute and chronic GvHD, respectively. Acute and chronic GvHD were graded according to clinical manifestations.[23–27] Transplant-related mortality was defined as death not related to disease recurrence or progression.

Statistical analysis

The results are expressed as medians and ranges (in parentheses) except for Figures 1–3 in which also the 10, 25, 75 and 90° percentiles are reported. Statistical analysis was carried out with the Student's *t*-test and the nonparametric test. Since patients rejecting their graft experienced higher TRM, only patients engrafted were included in these analyses. The following parameters were evaluated in multivariate Cox's analysis for the potential effect on

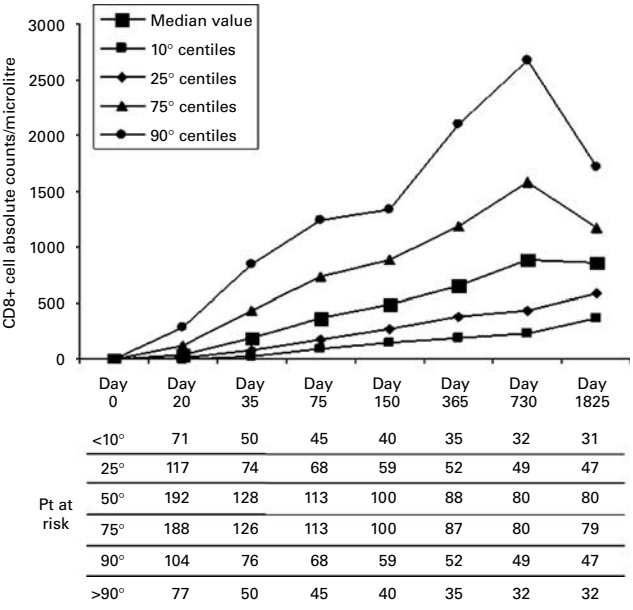

Figure 2 CD8+ cell recovery following allogeneic BMT. Median values and 10, 25, 75 and 90° percentiles are reported.

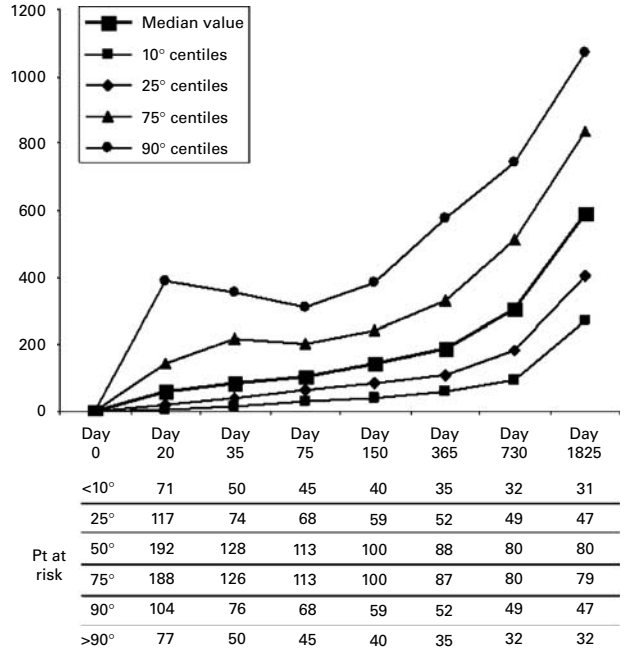

Figure 1 CD4+ cell recovery following allogeneic BMT. Median values and 10, 25, 75 and 90° percentiles are reported.

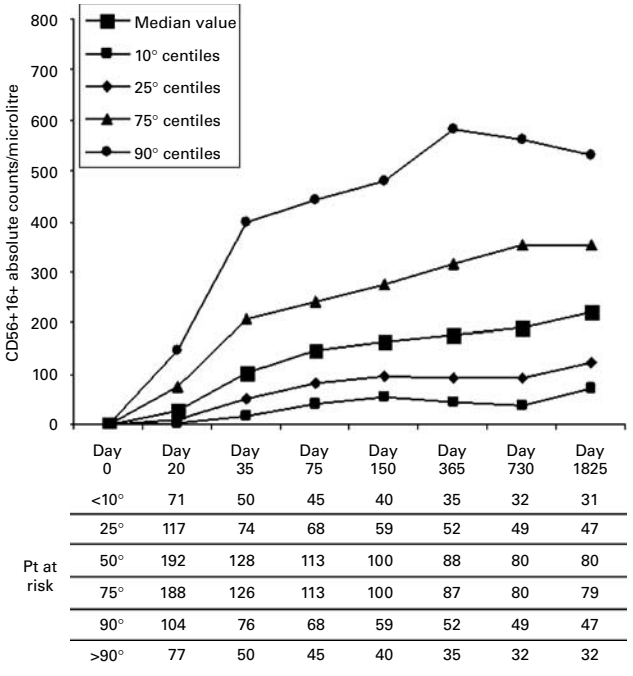

Figure 3 CD56+16+ cell recovery following allogeneic BMT. Median values and 10, 25, 75 and 90° percentiles are reported.

CD4+ cell recovery on day 35: donor type, cell dose, GvHD, recipient age, donor age, conditioning regimen and disease phase.

The CI of TRM according to the median number of CD4+, CD8+ and CD56+16+ on day 20, 35, 75 and 150 was reported as crude incidence curves and expressed as a percentage ±95% CI, in order to adjust the analysis for competing risk. The differences between groups were estimated by the log-rank test. For multivariate analysis a number of potential factors affecting TRM rate were evaluated: CD4+ cell number, donor type, GvHD, recipient age, donor age, conditioning regimen and disease phase.[28–30] The Number Cruncher software (NCSS, version 5.0; JL Hintze, Kaysville, UT) was used to perform the analysis (www.ncss.com).

Results

Lymphocyte reconstitution following HSCT

The first priority of this study was to gain an overall picture of T-cell reconstitution in this patient cohort by measuring the absolute number of circulating T-cell numbers. Total CD3+CD4+ (Figure 1), CD3+CD8+ (Figure 2) and CD3−CD56+CD16+ (Figure 3) cell numbers were measured at day 20, day 35, day 75, day 150, day 365, day 730 and day 1825. The median follow-up for the entire patient population is 724 days (25–4064); it is 1110 days (27–4064) and 363 days (25–2909), for the MSD and AD groups, respectively. Briefly, considering the entire patient population the median cell count rise above 200/μl after day 730 for the CD4+ and after day 75 for CD8+ cells. The median CD3−CD56+CD16+ cell count rise above 100/μl after day 35.

The donor type effect

CD4+ cell reconstitution was delayed in patients receiving an alternative graft rather than in MSD recipients until day 150. Comparing MSD to AD recipients, we found a statistical difference among the groups: day 35 CD4+ 123/μl (28–403) and 25/μl (2–121) for the MSD and AD groups, respectively ($P<0.004$), day 75 CD4+ 119/μl (5–1401) and 45/μl (9–138) for the MSD and AD groups ($P<0.003$) and day 150 with CD4+ 182/μl (57–630) and 63/μl (24–1233) for the MSD and AD groups, respectively ($P<0.0004$) (Figure 4a). When we compare separately the mismatch family and the unrelated donor recipients no significant differences could be observed in terms of CD4+, CD8+ and CD3−CD56+16+ cell recovery. We found how the median day 35 value for CD4+ cell/μl were 31 and 28 for the mismatch family and unrelated donor groups, respectively ($P=$NS), day 75 CD4+ cell/μl were 76 and 54 for the mismatch family and unrelated donor groups, respectively ($P=$NS), day 150 CD4+ cell/μl were 64 and 92 for the mismatch family and unrelated donor groups, respectively ($P=$NS). The CD4+ cell recovery behind day 150 was difficult to analyze in light of the low number of mismatch family donor patients. As the CD4+ cell recovery, when we compare the CD8+ or the CD3−CD56+CD16+ cell recovery at day 20, day 35, day 75 and day 150 no significant differences were found.

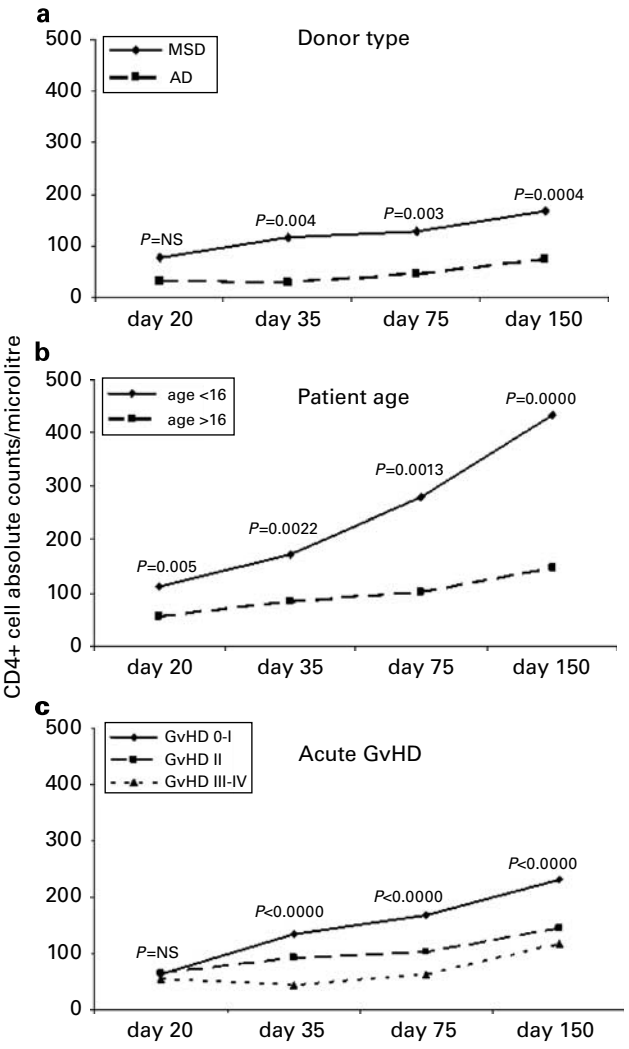

Figure 4 Univariate analysis of CD4+ cell recovery according to donor type (**a**) recipient age (**b**) and GvHD (**c**).

The recipient age effect

The patients were divided in two groups according to patient age younger or older than 16. Patients aged under 16 recovered CD4+ cells faster than older patients. Briefly day 20 CD4+ were 112/μl (21–282) for those younger than 16 and 56/μl (21–143) for older patients ($P=0.005$). Day 35 CD4+ were 173/μl (68–602) if recipients were younger than 16 and 84/μl (9–826) if they were older ($P=0.0022$). Day 75 CD4+ were 257/μl (63–420) if recipients were younger than 16 and 101/μl (53–360) if older than 16 ($P=0.0013$). Finally, day 150 CD4+ were 450/μl (219–640), 143/μl (25–629), ($P<0.0001$), respectively for recipients aged under or over 16 (Figure 4b).

The GvHD effect

The grading of acute GvHD (aGvHD) 0–IV was diagnosed according to clinical observation.[23] aGvHD was absent or

Table 2 Clinical outcome of patients undergoing allogeneic stem cell transplants

	MSD (%)	*AD (%)*
TRM	18	34
Relapse/progression	29	21
aGvHD $\geqslant 2$	47	63
cGvHD lim/extensive	33	47

Abbreviations: AD = alternative donor; aGvHD = acute GvHD (patients with evidence of donor engraftment who survived more than 14 days); cGvHD = chronic GvHD (patients with evidence of donor engraftment and alive on day 100); MSD = matched sibling donor; TRM = transplant related mortality.

graded I in 334 patients (49%). A total of 47 and 63% of patients transplanted with a MSD graft or AD recipients, respectively, developed GvHD grade II–IV. Chronic GvHD (cGvHD) was classified according to clinicopathologic finding.[24,25] It was absent in 433 patients (68%). A total of 33 and 47% of recipients of MSD graft or AD recipients developed cGvHD limited/extensive, respectively (Table 2). We analyzed the effect of GvHD on lymphocyte subsets reconstitution. For the CD4+ cell reconstitution following HSCT and day 35 time point, patients not developing aGvHD or graded I had CD4+ 135/μl (68–826), while patients having aGvHD II had CD4+ 91/μl (43–2226) and finally patients with aGvHD III–IV had CD4+ 45/μl (30–132), ($P<0.0000$). Day 75 CD4+ were 169/μl (68–826) for patients with no aGvHD or I, while patients having aGvHD II had CD4+ 104/μl (43–2226) and finally patients with aGvHD III–IV had CD4+ 63/μl (30–132), ($P<0.0000$), finally day 150 CD4+ were 232/μl (68–826) for patients with no aGvHD or I, 146/μl (43–2226) for patients having aGvHD II and finally patients with aGvHD III–IV had CD4+ 119/μl (30–132), ($P<0.0000$) (Figure 4c).

Multivariate analysis for CD4+ cell recovery as the endpoint

The donor type, the recipient age and the GvHD effects were analyzed in multivariate Cox's analysis for potential effects on day CD4+ cell recovery together with four other clinical factors: cell dose,[31] donor age, disease phase and conditioning regimen. In multivariate Cox's analysis for CD4+ cell recovery endpoint, donor type was a significant predictor (RR 3.45, CI 95% 2.9–4.7, $P=0.0001$) together with recipient age (RR 3.23, CI 95% 2.9–3.9, $P=0.003$), while the GvHD effect was lost in this multivariate analysis (Table 3).

Causes of death

One hundred seventy-one patients (23%) died of TRM between day 6 and 1630 after BMT. Seventy-six percent of all deaths occurred in the first year after BMT, and 60% within the first 6 months after transplant. The leading causes of death were infections (29%), GvHD (29%), interstitial pneumonia (27%) and liver failure and multiorgan failure (5%), hepatitis (5%), rejection (2%). In particular, TRM was 18 and 34% for MSD and AD transplant recipients, respectively. Relapse/progression was seen in 27% of patients undergoing allogeneic BMT: 29% of MSD and 21% of AD recipients.

Table 3 Multivariate analysis of factors influencing the probability of achieving a CD4+ cell count of >86/μl on day 35

Variables	*Baseline value*	*Compared value*	*RR*	*CI 95%*	P-*value*
Donor type	MSD	AD	3.45	2.9–4.7	0.0001
Cell dose	$>4.2\times10^8$/kg	$\leqslant 4.2\times10^8$/kg	1.08	0.8–1.2	0.61
Acute GvHD grading	0–I	II–IV	1.16	0.7–1.2	0.72
Recipient age	<16	$\geqslant 16$	3.23	2.9–3.9	0.003
Donor age	<35	$\geqslant 35$	1.16	0.8–1.4	0.32
Conditioning regimen	TBI yes	TBI no	1.37	1–1.6	0.91
Disease phase	Early	Advanced	1.09	0.6–1.4	0.76

Abbreviations: AD = alternative donor; MSD = matched sibling donor; RR = relative risk; TBI = total body irradiation.

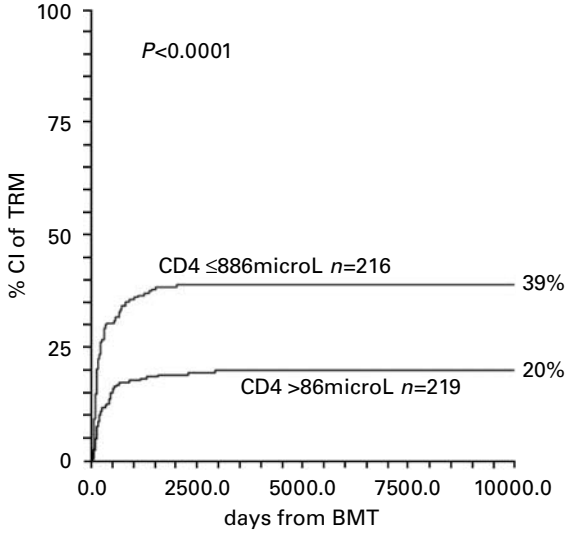

Figure 5 Cumulative incidence of TRM according to CD4+ cell count >86/μl on day 35 ($n=435$).

TRM and lymphocyte reconstitution

The median CD4+ cell count on day +35 for the entire patient population was 86/μl (range 22–198, $n=435$) and CI of TRM was significantly higher in patients not achieving that count: (20 vs 39%, $P<0.00001$) (Figure 5). The CI of achieving a CD4 count of 86/μl was 74%, it was 81% for MSD and 39% for AD ($P=0.0001$). In general, patients with low CD4+ count on day +35 had a higher risk of dying of infections (47 vs 24%, $P<0.0003$) (data not shown). CD8+ and CD56+16+ cell recovery did not correlate with different TRM risks.

When we carried out separate analysis on MSD and AD recipients and TRM risk as endpoint, according to median CD4+ cell count at day 35, we found how the higher CD4+ cell count retained a predictive effect for MSD (being TRM CI 20% compared to 44% for patients who did not reach CD4+ cell count >123/μl), this was not the case for the AD recipients (being TRM CI 32 and 50% for patients who reached CD4+ cell count above 25/μl on day

Table 4 Multivariate analysis of factors influencing the risk of transplant-related mortality

Variables	*Baseline value*	*Compared value*	*RR*	*CI 95%*	P-*value*
CD4+ cell number	>86/μl	⩽86/μl	1.97	1.7–2.5	0.0017
Donor type	MSD	AD	1.47	0.9–1.6	0.11
Acute GvHD grading	0–I	II–IV	1.59	1.4–1.7	0.0097
Recipient age	<16	⩾16	1.23	0.8–1.4	0.76
Donor age	<35	⩾35	1.2	0.9–1.4	0.13
Conditioning regimen	TBI yes	TBI no	1.09	0.7–1.3	0.49
Disease phase	Early	Advanced	1.11	0.7–1.3	0.44

Abbreviations: AD = alternative donor; MSD = matched sibling donor; RR = relative risk.
Early disease is first complete remission for acute leukemia or first chronic phase for chronic myeloid leukemia.
Advanced disease is for all other patients.

35). Furthermore, considering a more homogeneous patient population such as CML in first chronic phase, transplanted within 1 year from diagnosis, receiving a HSCT from MSD and BM as stem cell source, the TRM CI according to CD4+ >86/μl was 23% (CI 95% 18–30) compared to 45% (CI 95% 38–54) ($P = 0.004$) if CD4+ were less than 86/μl.

Multivariate analysis for TRM as the endpoint

In multivariate analysis on TRM, CD4+ cell count on day +35 was a very strong independent predictor (RR = 1.97; CI 95% 1.7–2.5, $P = 0.0017$), together with acute GvHD II–IV (RR = 1.59; CI 95% 1.4–1.7, $P = 0.0097$), while other clinical factors such as donor type, recipient age, donor age, conditioning regimen, cell dose and disease phase were not found to be predictors for TRM risk (Table 4).

Discussion

Our first task was to assess the lymphocyte recovery pattern in this large cohort of patients. Absolute numbers of CD8+ and CD56+16+ cells returned within normal ranges in a few months after allogeneic marrow transplant; by contrast, CD4+ cell recovery was depressed for up to 2 years following allogeneic transplantation as reported by Atkinson in a previous series.[8]

Univariate analyses show how three factors impact CD4+ cell recovery following BMT: donor type, GvHD and recipient age. In multivariate analysis donor type and recipient age maintained their predictive role for CD4+ cell recovery. Both these factors may be associated with GvHD, which is known to be increased in AD transplants and in older patients. Indeed, GvHD not only causes organ damage, but also induces a profound defect in the development of donor derived T cells, causing long lasting immune deficiency after transplant.[32]

Two series of patients who underwent allogeneic HSCT were analyzed for immune recovery. Rondelli *et al*., in a series of 32 allogeneic adult MSD HSCT, showed how patients developing aGvHD grade I or greater, had a lower number of both CD4+ and CD8+ cells, demonstrating that aGvHD does not selectively spare lymphocyte subsets.[33] By contrast, in a series of 91 patients, Fujimaki observed that aGvHD grade II–IV, did not affect CD4+, CD8+, CD4+CD45RA+ and CD4+CD29+ T cells, but rather CD56+16+ cells.[34] High dose immunosuppressive therapies given to treat GvHD are possibly an additional cause of impaired recovery.[35–38]

Recovery following allogeneic graft was also evaluated according to the recipients' age. In our series we found that patients under 16 years recovered CD4+ cell count earlier than older patients, confirming previous suggestions that age is a crucial factor determining the contribution of T-cell thymic output recovery post-HSCT. An age-related decline in T-cell function has indeed been described in various clinical settings. Moreover, in situations in which the thymic-dependent T-cell recovery is compromised, such as in patients with GvHD, the recovery of a normal diverse T-cell compartment could be delayed, possibly for many years following transplantation.[39] The lack of production of regenerating T cells (TREC+) suggests that GvHD itself or the immunosuppressive therapy, cause a shutdown in thymopoiesis. It is well documented that the thymus is a GvHD target organ and it has also become evident (during last 10 years) that thymic damage caused by alloreactive T cells may play a role in the pathogenesis of GvHD.[40]

In a second step we concentrated on early CD4+ cell recovery on day +35, which might be considered an early time point after engraftment. In univariate and multivariate analysis, patients with low CD4+ on day +35, had a significantly higher TRM CI. Univariate analysis shows, as expected, several factors correlating with TRM, such as AD, advanced disease, higher GvHD and recipient age. If we introduced in the Cox's model on TRM, also CD4+ cell recovery, this proved to be the strongest predictor of TRM (RR 1.97, $P = 0.0017$), together with higher GvHD. The same analysis was performed considering the CD8+ and CD56+16+ cell recovery following BMT, but no significant correlation with TRM was found. More importantly, the predictive value of the CD4+ cell recovery was retained when we analyzed separately MSD recipients (all diagnoses) or only patients given an MSD transplantation for CML in an early phase of disease. The cause of death which differed most in the two groups with counts lower or greater than 86/μl (median on day +35) was infections: therefore, patients with low CD4 counts die of infectious complications, with or without additional GvHD. Finally, our results agree with the Kim's study in which the 3 months CD4+ cell count was a predictive factor for both overall survival and TRM.[41]

Having shown that low CD4 counts on day +35 are predictive of TRM, the question is as follows: can we do something to modify this risk? Should we be more aggressive monitoring viral, fungal, bacterial infections? Should we run blood cultures also in afebrile patients, with the hope of identifying a pathogen and treat preemptively? Should we improve our diagnostic techniques with molecular biology also applied to blood cultures? Should we do what infectious disease specialist tell us we should not, that is prophylactic systemic antibacterial or antifungal therapy?

And finally, can we improve CD4 recovery by using cytokines such as IL7? We currently have no answers to these questions: however, having identified a group of patients at risk of lethal infections we can run prospective trials looking at different procedures with the aim of reducing TRM associated with infectious complications.

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