Original research paper

Quality of harvest and role of cell dose in unrelated bone marrow transplantation: An Italian Bone Marrow Donor Registry–Gruppo Italiano Trapianto di Midollo Osseo Study

Franca Fagioli[1], Paola Quarello[1], Simona Pollichieni[2], Teresa Lamparelli[3], Massimo Berger[1], Fabio Benedetti[4], Veronica Barat[1], Renato Marciano[2], Alessandro Rambaldi[5], Andrea Bacigalupo[3], Nicoletta Sacchi[2]

[1]Pediatric Onco-Hematology, Stem Cell Transplantation and Cellular Therapy Division, Regina Margherita Children's Hospital, Torino, Italy, [2]Ospedale Galliera, Italian Bone Marrow Donor Registry, Genova, Italy, [3]Division of Hematology, San Martino Hospital, Genova, Italy, [4]Department of Clinical and Experimental Medicine, Section of Hematology, University of Verona, Italy, [5]Division of Hematology, Research Foundation, Ospedali Riuniti di Bergamo, Bergamo, Italy

In this study, we investigated the factors affecting cell dose harvest and the role of cell dose on outcome. We analysed data from a cohort of 703 patients who underwent unrelated bone marrow transplantation facilitated by IBMDR in GITMO centers between 2002 and 2008. The median-infused cell doses is 3.7×10^8/kg, the correlation between the nucleated cells requested from transplant centers and those harvested by collection centers was adequate. A harvested/requested cells ratio lower than 0.5 was observed only in 3% of harvests. A volume of harvested marrow higher than the median value of 1270 ml was related to a significant lower infused cell dose (χ^2: 44.4; $P < 0.001$). No patient- or donor-related variables significantly influenced the cell dose except for the recipient younger age (χ^2: 95.7; $P < 0.001$) and non-malignant diseases (χ^2: 33.8; $P < 0.001$).

The cell dose resulted an independent predictor factor for a better outcome in patients affected by non-malignant disease ($P = 0.05$) while early disease malignant patients receiving a lower cell dose showed a higher risk of relapse ($P = 0.05$).

Keywords: Harvest, Cell dose, Unrelated bone marrow transplantation, Relapse

Introduction

Allogeneic bone marrow transplantation (BMT) from unrelated donors has become an effective treatment for patients with a wide variety of malignant and non-malignant diseases.[1–3]

Hematopoietic recovery, transplant-related mortality (TRM), relapse incidence (RI), and event-free survival (EFS), are influenced by patient-, disease-, and transplantation-related factors. Among those factors the number of infused nucleated cells (NCs) is included. In particular, several studies indicate that a higher NC dose improves hematopoietic recovery, overall survival (OS), EFS and reduces the risk of graft rejection, the occurrence of invasive fungal infection and cytomegalovirus disease, the TRM, the RI, and the graft-versus host disease (GVHD) incidence.[4–13]

The NC dose from bone marrow (BM) as stem cell source is a variable that can be controlled by clinicians since it has been recommended to transplant at least 2×10^8 NCs per kilogram of the recipient's body weight (BW).[14]

In 1989, the Italian Bone Marrow Transplant Group (Gruppo Italiano Trapianto Midollo Osseo (GITMO)) contributed in establishing the Italian Bone Marrow Donor Registry (IBMDR) to facilitate donor search for patients lacking an HLA identical sibling donor.[15] The IBMDR is a World Marrow Donor Association accredited Registry since 2007.

In order to analyse the harvest quality and the role of cell dose on transplant outcome we performed a

Correspondence to: Paola Quarello, Pediatric Onco-Hematology, Stem Cell Transplantation and Cellular Therapy Division, Regina Margherita Children's Hospital, Piazza Polonia, 94, Torino 10126, Italy.
Email: p.quarello@gmail.com

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retrospectively multicenter study on a large cohort of 703 patients who underwent unrelated BMT facilitated by the IBMDR in GITMO centers between 2002 and 2008.

Methods

Donor and patient features

We analysed the first BM harvest performed on 703 donors in 39 IBMDR collection centers. The transplantation procedures were done in 47 GITMO centers between January 2002 and December 2008. The donor search and selection criteria were conducted according to IBMDR standard.[16]

The donor and patient features are reported in Table 1.

Molecular-based HLA A, HLA B, HLA C, HLA DRB1, HLA DQB1 typing of the donor/recipient pairs was done according to IBMDR criteria and performed in European Federation for Immunogenetics accredited laboratories.

Table 1 Clinical data

No. of patients	703
Median age at transplantation, years (range)	27 (0–64)
Patients gender (male/female)	404/299
Median patient weight, kg (range)	60 (6–121)
Disease, no. of patients (%)	
Malignant disease	587 (83.5)
ALL	191
AML	194
LPDs	89
Myelodisplasia	56
CML	52
Solid tumors	5
Non-malignant disease	116 (16.5)
Thalassemia	47
Inborn error	36
AA	28
Hemophagocytic lymphohistiocytosis	5
Disease state, no. of patients (%) (leukemia patients only)	437 (100)
Early	161 (37)
Advanced	257 (59)
NA	19 (4)
Previous BMT	
No	580 (83)
Yes	116 (16)
NA	7 (1)
Median time between diagnosis and BMT, days (range)	
Non-malignant disease	890 (78–12 649)
Malignant disease	507 (89–7952)
Median time follow-up, days (range)	829 (1–3292)
Median follow-up for deceased patients	146 (1–2426)
Median follow-up for surviving patients	1738 (32–3292)
Median donor age, years (range)	35 (19–53)
Donor gender (male/female)	480/186 (37 NA)
Median donor weight, kg (range)	73 (50–125)
HLA typing	
10/10	301 (43%)
9/10	239 (34%)
8/10 or less	133 (19%)
NA	30 (4%)
Recipient–donor sex match, no. of patients (%)	
Male/female	112 (16)
Others	591 (84)

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; LPDs, lymphoproliferative disorders; CML, chronic myeloid leukemia; AA, severe aplastic anemia; NA, not available.

Conditioning regimen and GVHD prophylaxis

A total of 541 patients (77%) received a myeloablative regimen while 162 patients (23%) had a reduced intensity conditioning. Total body irradiation was used in 52% of cases (9.9–12 Gy). Among patients receiving a reduced intensity conditioning preparative regimen, 94% were affected by malignant disease and 6% by a non-malignant disease.

Acute and chronic GVHD (aGVHD, cGVHD) were reported according to established criteria.

Data collection and statistical analysis

Essential data regarding all donor–recipient pairs and information concerning the harvesting procedure were obtained by the IBMDR data center. Each GITMO transplant centers updated patient clinical data using standardized forms at the time of transplantation, at day +100, at 6 months, and annually afterward. All data were checked and validated before statistical analysis through the sending of an appropriate database resent to transplant centers to confirm data and to rescue all missing data. All patients or their legal guardians signed the appropriate informed consent form previously approved by the local ethic committee or the Institutional Review Board.

The cell dose was defined as infused total NCs. The first analysis was conducted to define factors affecting the cell dose harvest. For the statistical analysis, continuous variables were categorized as follows: each variable was first divided into four categories at the twenty-fifth, fiftieth, and seventy-fifth percentiles. If the relative event rates (the ratio of the observed number of events to the expected number of events in the category) in two or more adjacent categories (and the median time to events) did not differ, those categories were grouped. If no clear pattern was observed for the primary outcomes, the median was taken as the cutoff point. The differences were calculated according to χ^2 test. We then analysed the cell dose effect on OS, EFS, TRM, RI, aGVHD, and cGVHD incidence. The impact of cell dose on patient outcome was first carried out on all cohorts and later the analysis was performed separately on subgroups based on disease type and malignant disease stage.

OS was calculated from transplantation to death due to any cause. EFS was defined as the probability of being alive without recurrence of disease: events were the death in remission, the relapse, the graft failure, whichever occurred first. TRM was defined as death due to causes unrelated to underlying disease. Relapse was defined on the basis of morphological evidence of tumor cells in BM or other sites.

For solid tumor patients the progression was defined according to Response Evaluation Criteria in Solid Tumors.[17]

Engraftment was defined as the first of three consecutive days with an absolute neutrophil count $\geq 0.5 \times 10^9$/l. The patients were censored at the time of relapse, death, or last follow-up. OS and EFS were calculated according to the Kaplan and Meier method, while the aGVHD and cGVHD occurrence, as well as TRM and RI were expressed as cumulative incidence curves, in order to adjust the analysis for competing risks. The significance of differences between OS, EFS was estimated by the log–rank test (Mantel–Cox) as well as differences between TRM and RI curves by Gray's test.[18]

The competitive events for aGVHD and cGVHD were death in remission and relapse. The competitive event for RI was the death in remission, and on the contrary the RI was the competing event for calculating the TRM incidence.

All variables having a P value less than 0.1 in univariate analyses were included in a multivariate analysis performed using the Cox proportional regression model. P values less than 0.05 were considered to be statistically significant.

All statistical analyses were performed with SPSS 18.0 (Chicago, IL, USA), except for competitive risk analysis that was carried out by NCSS 2004 software for Windows.

Results

Bone marrow collection

The median harvest number performed by each IBMDR collection center between 2002 and 2008 was 10 (range 1–108). Eighty-five percent of BM collections were done in centers that performed more than 10 harvests during the study period.

The median nucleated requested cells were 4×10^8/kg of recipient BW (range 2–5), the median nucleated harvested cells were 4.1×10^8/kg (range 1–27), and the median nucleated infused cells were 3.7×10^8/kg (range 0.15–15). The median harvested/requested cells ratio was 0.97 (range 0.28–3.34). A ratio lower than 0.5 was observed only in 3% of harvests. This ratio did not reflect a low efficiency of the harvesting, in all cases the requested cells were higher than 3×10^8/kg of recipient BW (range 3–8.35) and the collected cells were lower than 1.5×10^8/kg of recipient BW only in two harvests.

The median harvests volume including anticoagulant was 1270 ml (range 270–2660 ml). The median BM collected for donor BW was 17 (range 3–32 ml) and 22 ml for recipient BW (range 10–100 ml), respectively. Data about anticoagulant were available on 564/703 harvests (80%). Heparin was used as anticoagulant for 374 harvests (66%), sodium citrate with citric acid anhydrous dextrose, and heparin and citric acid anhydrous dextrose only for 147 (26%) and for 43 (8%), respectively.

Data regarding manipulation were available for 552/703 harvests (78.5%). BM manipulation (including red blood cell and/or plasma removal and $CD34^+$ selection) has been performed in 312 out of 552 patients. Specifically, red blood cell removal alone has been performed in 96 (31%), plasma removal alone in 161 (52%), plasma and red blood cell removal in 46 (15%), and $CD34^+$ selection in six (2%) grafts before infusion. Harvested BM was

Table 2 Harvest cell dose according to clinical data

		<2.8 (×10^8/kg)	2.8–3.7 (×10^8/kg)	3.7–5 (×10^8/kg)	>5 (×10^8/kg)	X^2	P
Harvest number	> 10 (n = 470)	109	121	120	120	1.14	0.76
by Center	< 10 (n = 85)	23	18	21	23		
Harvest volume*	< 1270 ml (n = 282)	60	49	70	103	44.4	<0.001
	> 1270 ml (n = 278)	74	94	71	39		
Graft	Yes (n = 301)	87	67	65	82	14	0.002
manipulation	No (n = 239)	42	72	69	56		
Donor age	< 35 years (n = 301)	77	76	69	79	1.4	0.7
	≥ 35 years (n = 303)	70	79	80	74		
Donor gender	Male (n = 429)	97	111	112	109	3.1	0.37
	Female (n = 175)	50	44	37	44		
Donor weight	<73 kg (n = 280)	64	74	68	74	0.75	0.86
	≥73 kg (n = 315)	80	81	78	76		
Patient age	<27 years (n = 303)	39	62	79	123	95.7	<0.001
	≥27 years (n = 301)	108	93	70	30		
Patient gender	Male (n = 348)	94	90	85	79	4.69	0.19
	Female (n = 256)	53	65	64	74		
Patient weight	<60 kg (n = 256)	26	37	72	121	151.3	<0.001
	≥60 kg (n = 311)	110	108	71	22		
Disease	Malignant (n = 513)	135	144	124	110	33.8	<0.001
	Non-malignant (n = 91)	12	11	25	43		
Disease status	Early (n = 141)	43	38	33	27	2.5	0.47
	Advanced (n = 224)	56	61	50	57		

*Including anticoagulant.

infused in the recipient within 24 hours in almost all patients (92%, 527/576).

Factors affecting cell harvest

We analysed the influence of harvest-, donor-, and patient-related factors on harvested cell dose. Patients were first stratified into four groups according to percentiles of infused NCs (<25th percentiles: $<2.8\times10^8$/kg, 25–50th: $\geq2.8<3.7\times10^8$/kg, 50–75th: $\geq3.7<5\times10^8$/kg, ≥75th: $\geq5\times10^8$/kg) (Table 2).

Among the harvest-related factors a lower harvest volume (<1270 ml) corresponded to a higher infused cell dose ($P<0.001$), while the graft manipulation had a detrimental effect on infused cell dose ($P=0.002$) (Table 2). Specifically, a statistical significant loss of NCs/kg recipient BW was observed after red cell removal (mean harvested NC $=4.85\times10^8$/kg BW versus mean infused NC $=3.54\times10^8$/kg BW, $P=0.0003$) and red cell combined to plasma removal (mean harvested NC $=6.32\times10^8$/kg BW versus mean infused NC $=4.33\times10^8$/kg BW, $P=$

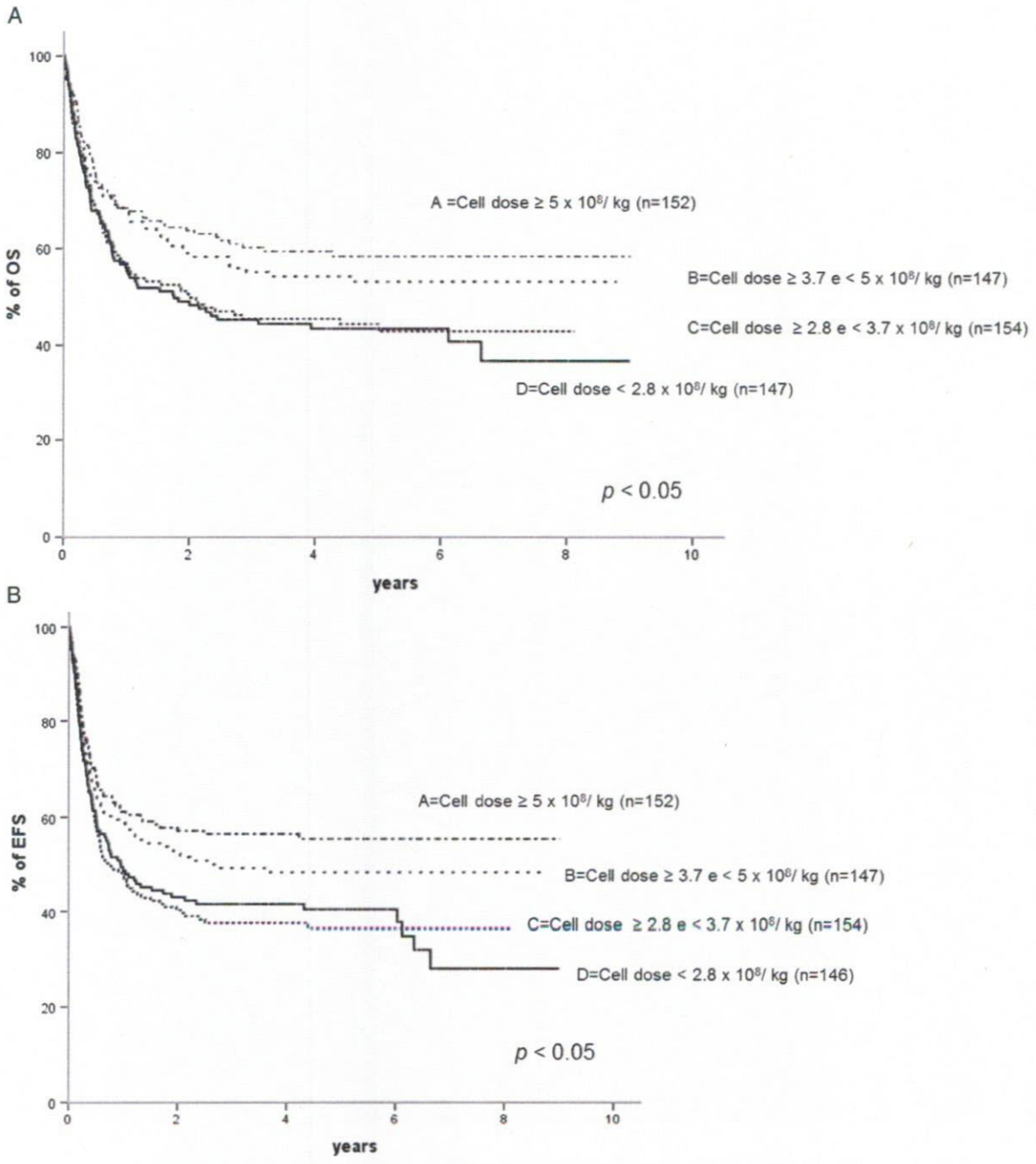

Figure 1 Kaplan–Meier estimate of OS and EFS after BMT according to bone marrow cell dose infused in four categories (percentiles).(A) *P* values in the log-rank test are as follows: A versus B, $P=0.78$; A versus C, $P=0.05$; A versus D, $P=0.006$; B versus C, $P=0.08$; B versus D, $P=0.01$; C versus D, $P=0.38$. (B) *P* values in the log-rank test are as follows: A versus B, $P=0.89$; A versus C, $P=0.08$; A versus D, $P=0.006$; B versus C, $P=0.03$; B versus D, $P=0.004$; C versus D, $P=0.27$.

0.02). No differences between harvested and infused NC was observed in the case of plasma removal (mean harvested NC = 4.7×10^8/kg BW versus mean infused NC = 4.5×10^8/kg BW, $P = 0.2$).

Regarding patient-related factors we found that both younger age (<27 years) and lower weight (<60 kg) were related to a higher infused cell dose ($P < 0.001$).

Patients affected by non-malignant disease received a higher cell number ($P < 0.001$). Donor-related factors (age, gender, weight) did not impact on infused cell dose (Table 2).

Cell dose and outcome

We previously analysed the influence of cell dose on OS and EFS according to percentiles as shown in Fig. 1. We found that the median value (3.7×10^8/kg) gave the greatest discrimination for the end points (Fig. 2). The actuarial 5-year OS and EFS were significantly different in patients receiving high or low cell dose: OS 55.8 ± 3 versus 43.1 ± 3% ($P = 0.002$) and 52 ± 2.9 versus 38.4 ± 2.9% ($P = 0.001$), respectively.

The TRM and the RI resulted lower in higher cell dose group even if not statistically significant (20.4 ± 2.3 versus 26.5 ± 2.5%, $P = 0.09$; 23.8 ± 2.9 versus 30.1 ± 2.6%, $P = 0.06$).

The cell dose did not influence neutrophil recovery at day +100 (high cell dose group 92 ± 1.6 versus 91 ± 1.7%, $P = 0.15$).

The incidence of aGVHD and cGVHD was not different in the two groups of patients and thus not related to the cell dose.

The other factors other than cell dose affecting outcome are reported in Table 3.

Multivariate analysis on outcome

The cell dose was then analysed in multivariate Cox analysis for potential effect on outcome together with the other clinical factors that resulted significantly in univariate analysis.

When we considered the entire patient population, the cell dose effect on patient outcome was not confirmed, while the multivariate Cox analysis showed a statistical association between younger patient age (OS: RR 0.56, 95% CI 0.44–0.71, $P < 0.001$; EFS: RR 0.56, 95% CI 0.44–0.70, $P < 0.001$), non-malignant diseases (OS: RR 0.40, 95% CI 0.26–0.70, $P < 0.001$; EFS: RR 0.43, 95% CI 0.28–0.68, $P < 0.001$), and a 10/10 HLA-matched donor (OS: RR 0.67,

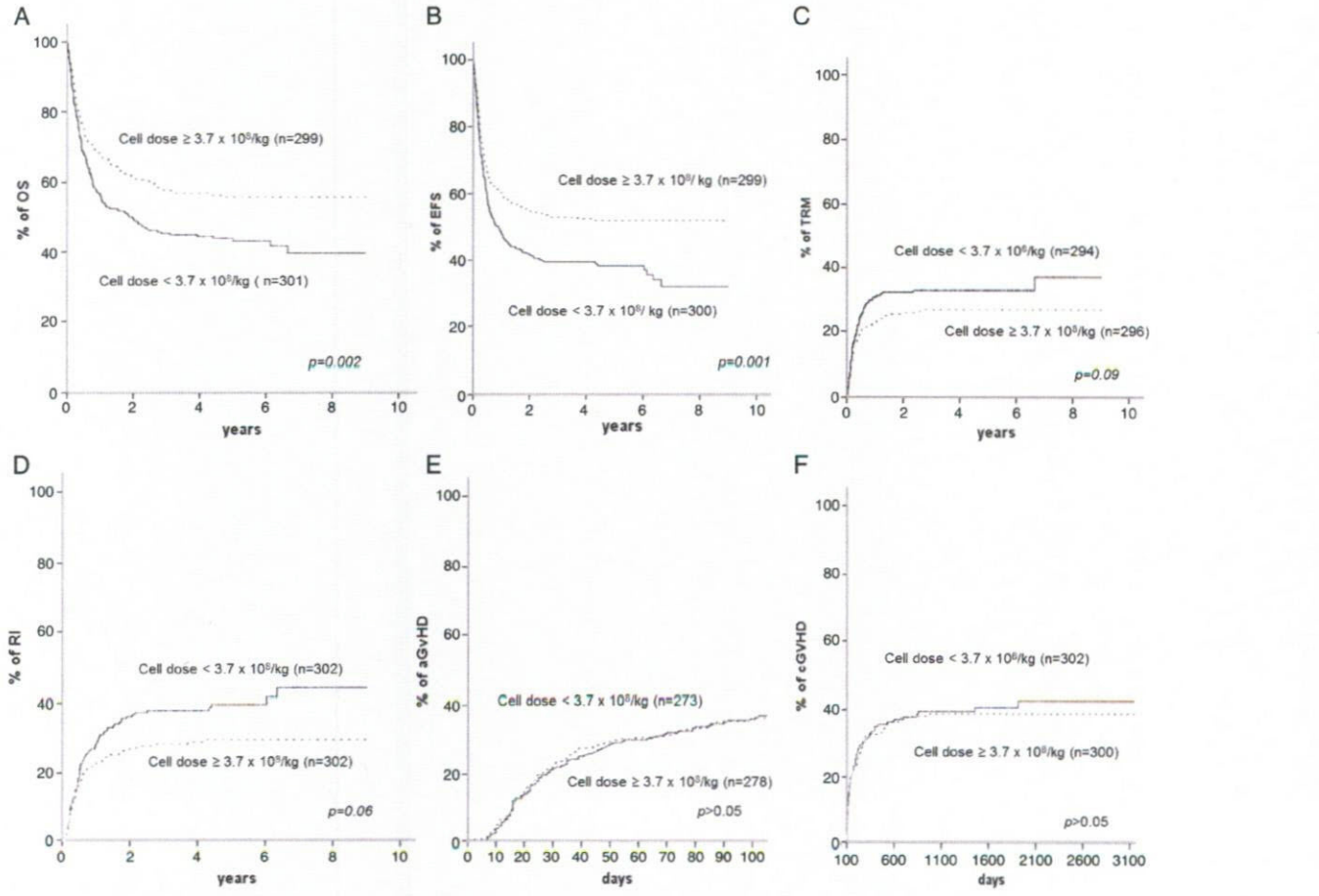

Figure 2 Kaplan–Meier estimate of (A) OS, (B) EFS, cumulative incidence of (C) TRM, (D) RI, aGVHD (E), and cGVHD (F) incidence, according to bone marrow cell dose infused.

Table 3 Harvest-, donor-, and patient-related factors according to bone marrow cell dose infused

		OS (%)	EFS (%)	TRM (%)	Relapse (%)
Overall		50 ± 2	45.4 ± 1.9	26.5 ± 1.6	25.2 ± 1.7
Harvest-related factors					
Interval between harvest and infusion	0 days (n = 462)	50.6 ± 2.3	46.2 ± 2.2	28.9 ± 2.2	34.5 ± 2.4
	1day(n = 39)	45 ± 7.4	37.4 ± 8.2	30.1 ± 7.2	40.8 ± 10
	P	0.23	0.51	0.12	0.61
Graft manipulation	Yes (n = 310)	53.6 ± 2	49.7 ± 2.9	26.6 ± 2.7	29.9 ± 3
	No (n = 237)	47.3 ± 3	42.1 ± 3.3	31.6 ± 3.4	38.6 ± 3.8
	P	0.24	0.11	0.36	0.36
Donor-related factors					
Age	<35 years (n = 341)	50.5 ± 3	48.5 ± 3.9	21.8 ± 3.2	32.6 ± 3
	≥35 years (n = 355)	50.6 ± 4	44.1± 4.2	21.9 ± 3.2	36 ± 3
	P	0.58	0.53	0.44	0.65
Gender	M (n = 479)	50.4 ± 2	46.3 ± 2.3	27.6 ± 2.2	36.6 ± 2.6
	F (n = 186)	50.5 ± 3.7	45.2 ± 3.7	33.5 ± 3.8	29.1 ± 3.6
	P	0.51	0.84	0.39	0.17
Weight	≥73 kg (n = 361)	51.8 ± 3	46.7 ± 3	27.4 ± 2.8	33.4 ± 3
	<73 kg (n = 324)	48.6 ± 3	45.4 ± 3.3	31.1 ± 3.3	34.9 ± 3.1
	P	0.49	0.67	0.34	0.56
HLA compatibility	10/10 (n = 316)	55.6 ± 3	49.4 ± 3	25 ± 2.7	33.1 ± 3
	Others (n = 379)	45.5 ± 2.6	42.3 ± 2.6	33.5 ± 2.6	35.7 ± 3
	P	0.01	0.02	0.015	0.4
Gender match D/R	F/M (n = 117)	43.3 ± 4.7	40.3 ± 4.6	35 ± 5	33.7 ± 5.2
	Others (n = 580)	51.3 ± 2.2	46.4 ± 2.1	29 ± 2	34.5 ± 2.3
	P	0.11	0.24	0.3	0.8
Patient-related factors					
Age	<27 years (n = 346)	62 ± 2.7	58.1 ± 2.7	15.2 ± 1.9	27.3 ± 2.6
	≥27 years (n = 350)	38.1 ± 2.7	32.8 ± 2.6	34.5 ± 2.6	23.1 ± 3.4
	P	<0.001	<0.001	<0.001	0.1
Gender	M (n = 398)	47.3 ± 2.6	43.3 ± 2.5	30.2 ± 2.5	37.8 ± 2.9
	F (n = 297)	53.4 ± 3	48.2 ± 2.9	29.4 ± 2.9	29.9 ± 3.1
	P	0.24	0.35	0.90	0.07
Weight	≥60 kg (n = 314)	38.3 ± 2	33.7 ± 2.7	28.4 ± 2.5	31.9± 3.5
	<60 kg (n = 260)	64.1± 3	59.8 ± 3.1	15.8 ± 2.7	20.8 ± 3
	P	<0.001	<0.001	<0.001	<0.001
Disease	Malignant (n = 583)	44.4 ± 2.1	40.9 ± 2.1	24.6 ± 1.6	—
	Non-malignant (n = 113)	80.4 ± 3.8	75.7 ± 4.1	16.8 ± 3.6	—
	P	<0.001	<0.001	0.04	—
Disease status	Early (n = 161)	64.6 ± 3	56.3 ± 4	17.5 ± 3	25 ± 3.4
	Advanced (n = 257)	35.5 ± 3	32.1 ± 3	25.5 ± 2.7	37.6 ± 3
	P	<0.001	<0.001	0.05	0.04
Infused nucleated cells	<3.7×10^8 (n = 301)	43.1 ± 3	38.4 ± 2.9	26.5 ± 2.5	30.1 ± 2.6
	≥3.7×10^8 (n = 299)	55.8 ± 3	52 ± 2.9	20.4 ± 2.3	23.8 ± 2.9
	P	0.002	0.001	0.09	0.06

M, male; F, female; D/R, donor/recipient.

95% CI 0.53–0.85, $P = 0.001$; EFS: RR 0.77, 95% CI 0.58–0.90, $P = 0.01$) with better OS and EFS.

Subgroup analyses

Because this analysis was retrospective, the patient population was quite heterogeneous. Therefore, analyses were done on a more homogeneous patient subgroup to investigate if the cell dose had an independent variable role.

Malignant disease

The multivariate analysis did not show significant association between cell dose and outcome in the cohort of patients affected by malignant disease.

Considering patients with early status malignant disease we observed that the actuarial 5-year OS, EFS, and TRM results were not influenced by cell dose. Instead, in this group of patients we observed a role of cell dose on RI. In fact, patients receiving a cell dose <3.7×10^8/kg showed a significant higher risk of relapse (30.8 ± 5.1 versus 16.6 ± 4.8%, $P = 0.05$).

Among patients with advanced disease, the actuarial 5-year OS, EFS, TRM, and RI were not related to infused cell number. We did not find any correlation between cell dose and neutrophil recovery, aGVHD, and cGVHD incidence. Considering specific diseases (ALL, AML, CML, LPDs, MDS) we did not observe any impact of cell dose on EFS, TRM, and RI (Table 4).

Non-malignant disease

The actuarial 5-year OS and EFS were 80.4 ± 3.8 and 75.7 ± 4.1%, respectively. Forty-seven percent of these patients received more than 5×10^8/kg NCs while 14% of the patients had less than 2.8×10^8/kg. In the 26% the cell dose was included between 3.7×10^8 and 5 ×

Table 4 EFS, TRM, and RI in malignant disease according to infused cell dose

Median infused cell dose	ALL		AML		CML		LPDs		MDS	
	≤4	>4	≤3.1	>3.1	≤3.3	>3.3	≤3.6	>3.6	≤3.4	>3.4
EFS	33.7%	46.5%	28.2%	36.1%	47.4%	66.7%	29.1%	38.1%	46.2%	51.2%
95% CI	(28.4–39)	(40.9–52.1)	(21–35.4)	(30.4–42)	(36.4–58.4)	(56.7–76.7)	(22.1–36)	(30–46)	(36.4–56)	(41.2–61)
P	0.11		0.86		0.09		0.5		0.47	
TRM	31.6%	19%	25.8%	24.6%	21.7%	9.5%	26.8%	22.8%	32%	25%
95% CI	(22.8–43.7)	(12.2–29.6)	(18–37)	(16.7–36.4)	(10–47.2)	(2.5–35.5)	(16.1–44.4)	(12.4–42)	(18–56)	(12.5–49)
P	0.06		0.71		0.21		0.73		0.58	
RI	31.6%	32.1%	32.9%	32.4%	30.4%	23.8%	34.1%	28.5%	20.1%	20.8%
95% CI	(22.8–43.7)	(23.5–43.8)	(24.3–44.6)	(23.5–44.8)	(16.4–56.4)	(11–51.1)	(22.3–52.2)	(16.9–48)	(9.1–43)	(9.5–45.4)
P	0.78		0.88		0.45		0.62		0.96	

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; LPDs, lymphoproliferative disorders; MDS, myelodisplasia.

10^8/kg and in the 13% between 2.8×10^8 and 3.7×10^8/kg. The multivariate analysis on OS and EFS carried out on patients affected by non-malignant disease evidenced the cell dose $>5 \times 10^8$/kg as an independent predictor factor (RR 0.66, 95% CI 0.40–0.99, $P = 0.05$). We did not find any correlation between cell dose and neutrophil recovery, aGVHD, and cGVHD incidence.

Discussion

The aims of this study were to analyse the quality of harvests and to evaluate the impact of NC dose on outcome in 703 patients who received unrelated BMT between 2002 and 2008.

The harvest quality, expressed as cell density of NCs (cell dose), depends on several factors donor-, patient-, and harvest-related.

Differently from donor- and patient-related factors, the BM cell dose is a variable that can be controlled by clinicians since it has been recommended to transplant at least 2×10^8 NCs per kilogram of the recipient's BW.

In our cohort, we highlighted an adequate correlation between the NCs requested from transplant centers and the NCs harvested by collection centers. Interestingly, a harvested/requested cells ratio lower than 0.5 was observed only in 3% of harvests. This ratio did not reflect a low efficiency of the harvesting, in fact in all cases the requested cells were higher than 3×10^8/kg of recipient BW (range 3–8.35) while the collected cells were lower than 1.5×10^8/kg of recipient BW only in two harvests.

We also observed that harvest volume and collection technique are found to have a significant impact on the NC dose. Previous studies showed that the harvest volume is inversely related to the cell density of total NC in the marrow harvest most likely due to a peripheral blood contamination.[19–21] In our cohort, we found that a volume of harvested marrow lower than the median value of 1270 ml was statistically related to a higher infused cell dose ($\geq 3.7 \times 10^8$/kg, $P < 0.001$) in keeping with other studies. In order to reduce the peripheral blood contamination and thus the harvest volume, a consolidated strategy is based on multiple BM aspirations of small quantities instead of multiple large aspirates.[21, 22]

A variety of manipulations can be performed on marrow for transplantation. Allogeneic transplantation may require red blood cell or plasma removal if the transplant is ABO incompatible. In our cohort, a graft processing has been performed in more than 50% of marrow transplantations. We observed that the red cell combined to plasma removal or red cell removal alone had a significant negative impact on infused NC dose. On the contrary plasma depletion alone was not associated with changes in infused cell dose. These data are useful to physicians since the expected effect of processing on graft cell dose is necessary to plan the optimal volume of BM cells to be collected.

Apart from harvest-related variables, several studies analysed the influence of donor features on BM harvest quality.[19,18,23] We carried out the analysis of donor features (age, gender, and weight) on harvest quality and could not identify any influence of these factors on BM cell dose.

Many studies reported that higher cell dose improves OS rates,[5,7,18,24,25] however effects of cell dose on relapse and TRM rates were not consistent among studies probably because of differences in disease, stages, and transplant procedure.

In our large cohort we found a significant role of cell dose on OS and EFS improvement and on RI reduction. A trend for lower TRM in patients receiving a higher dose of NCs was also observed. The effect of cell dose analysed together with other known predictive factors (younger age, non-malignant disease, 10/10 HLA-matched donor) was not confirmed to be an independent prognostic factor on outcome. Thereafter, when we considered a

more homogenous patient population such as the non-malignant disease group, the cell dose impact on outcome was recovered also in multivariate analysis.

Despite the heterogeneity of BM failure syndrome, hemoglobin disorders, and inborn errors, many of the challenges to successful HSCT are similar. For non-malignant diseases the main problems encountered with HSCT were the high incidence of graft failure and GVHD.[26–28] Only a few reports regarding the influence of nucleated infused cell dose on outcome in these patients are present in literature.[9]

A relevant finding of this study is the demonstration that a cell dose higher than 5×10^8/kg had a significant predictor role for better outcome both on OS and EFS in this subgroup of patients without increasing the incidence of GVHD.

Previous reports have shown that a higher marrow cell dose reduced the severity of aGVHD.[5,12,29] In our cohort, we did not observe any association between the infused cell density and the incidence of aGVHD or cGVHD. Moreover, we confirmed that the occurrence of 0-1 acute or limited cGVHD had a protective role in terms of OS, EFS, and TRM.

Effects of cell dose on relapse rates were controversial. Rocha *et al.* [8] and Barret *et al.* [6] showed that cell dose was associated with decreased relapse rates, whereas several studies including various diseases did not show any effect of cell density on relapse rates.[5,7,18]

Interestingly, our results showed a specific role of infused cell dose in the subgroup of patients affected by early status malignant disease. In fact, in this group we observed a trend of higher risk of relapse in patients receiving a cell dose lower than the median value of 3.7×10^8/kg ($P = 0.05$). No association was observed between high cell dose and aGVHD incidence.

This observation is consistent with Inamoto *et al.* [30] and Rocha *et al.* [8] data that showed lower relapse rates in early-stage diseases not associated with higher incidences of aGVHD.

Moreover, it has been demonstrated that a GVL effect works more efficiently for patients with a lower disease burden than for those affected by advanced status disease.[31,32] Therefore, it is reasonable that decreased relapse rates associated to a higher cell dose was observed in early-stage diseases only.

Rocha *et al.* [8] speculated that the reason why a higher cell dose decreased relapse without increasing GVHD relies on the fact that there are cell subtypes other than T cells that could influence relapse.[33] Unfortunately, as we did not have data about graft composition during the study period we could not confirm this intriguing hypothesis. Further studies quantifying BM subpopulations in the graft and their role on relapse are warranted.

Conclusion

In conclusion, we have observed a very good efficiency of collection centers in respect to transplant centers' requests. The harvest-related factors in terms of harvest volume and graft manipulation had a strong impact on infused cell dose. This study also shows that the marrow cell dose is an important factor influencing outcome in non-malignant diseases and RI in early-stage malignant diseases. Therefore, it seems reasonable, on the basis of these findings, to harvest more cells during the collection procedure in patients affected by non-malignant disease and in patients affected by early-stage malignant disease.

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