Osteonecrosis after allogeneic stem cell transplantation in childhood. A case-control study in Italy

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A case-control study was conducted among Italian children treated with a stem cell transplant (SCT). Cases (n=43) were allogeneic recipients with osteonecrosis, and the controls (n=129) were matched to the corresponding cases on the basis of survival, SCT center and date of transplant. Univariate analysis showed that older age at SCT (OR 1.39; 95% CI 1.24-1.57), total body irradiation (TBI) (OR 5.73; 95% CI 2.38-13.83), chronic graft-versus-host disease (GvHD) (OR 4.31; 95% CI 2.05-9.07), and duration of steroid treatment after SCT (OR 1.118; 95% CI 1.034-1.209) were statistically correlated with osteonecrosis. However, multivariate analysis revealed that the only factors that were significantly associated with osteonecrosis were older age at SCT (p=0.0001), TBI (p=0.001) and chronicGvHD (p=0.001).

Key words: osteonecrosis, allogeneic stem cell transplantation, GvHD, steroid.

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Osteonecrosis is a clinical condition that is frequently reported following stem cell transplantation (SCT), but only few studies have examined this complication in children.[1-7] Older age at transplant is considered an important risk factor for osteonecrosis, as is allogeneic SCT, acute and/or chronic graft versus host disease (GvHD), type of primary disease, and total body irradiation (TBI).[1-3,5,6,8,9] Steroid administration has also been indicated as a possible risk factor in many reports, but only few studies actually analyzed the effects of cumulative dose or duration of treatment and controversial results were obtained.[2-4,6,8,10] We analyzed the effects of cumulative dose and duration of steroid treatment, as well as of other possible risk factors for osteonecrosis in a group of Italian children undergoing allogeneic SCT.

Design and Methods

A 1:3 case-control study design was used, involving the allogeneic SCT recipients who were enrolled in the Italian Association of Pediatric Hematology and Oncology (AIEOP) - SCT Registry, and who were treated between 1989 and 2002 at any one of the AIEOP centers. Cases were defined as children who developed clinically and radiologically documented osteonecrosis after allogeneic SCT. Patients affected by metabolic storage diseases or osteopetrosis, as well as those who developed osteonecrosis prior to the first or following a second SCT were excluded.

Matching criteria

Controls had to be allogeneic recipients without osteonecrosis. They were matched to the corresponding case according to specific characteristics with the following order of importance: survival equal to or greater than the interval between transplant and osteonecrosis; AIEOP - SCT center; date of transplant. Data concerning demographics, type of primary diagnosis, date and type of transplant, conditioning regimen, and GvHD (grade, duration and treatment) were retrieved from the AIEOP - SCT Registry for each study subject. Further information was also collected regarding radiation (field, cumulative dose, and fractioning) and on the administration of steroids, either before or after SCT. Steroid therapy before SCT was coded as either administered continuously or not administered continuously for at least 3 weeks, while the cumulative dose that was received after SCT, if given, was calculated and converted to the equivalent dose of methylprednisolone. Data regarding any event or treatment after SCT among controls were censored at the time interval after SCT when the corresponding case was diagnosed with osteonecrosis.

This retrospective study was approved by the AIEOP-SCT board, and data were collected anonymously. Moreover, all the SCT patients or their guardians had previously signed a consent form allowing us to use their data for clinical research purposes. The procedures we followed were in accordance with the our institution's ethical standards and with the Helsinki Declaration.

According to Italian guidelines, no other specific informed consent was required.

Standard peri-transplant and post-transplant management

In general, radiotherapy, either TBI or thoracoabdominal irradiation (TAI), was only used in the conditioning regimen to treat malignancies. TBI was administered either in three fractions of 330 cGy/day (total dose 990 cGy), or in six fractions of 200 cGy /twice a day (total dose 1200 cGy), while TAI was administered in a single dose of 600 cGy. GvHD was usually treated by steroids associated with immunosuppressive drugs, which were then tapered according to the patient's clinical conditions.

Statistical analysis

Data are described as absolute and relative frequencies for categorical variables, and as means with standard deviation (SD) and medians for continuous variables. Univariate analysis was initially carried out to determine which risk factors were significantly associated with the risk of osteonecrosis. Logistic regression analyses were used for each variable, and the results are reported as odds ratio (OR) with their 95% confidence intervals (CI). The absence of exposure to the factor or the variable that was less likely to be associated with the risk of osteonecrosis was used as the reference for each analysis we performed. Once the effect of the underlying disease had been evaluated, and the results showed that the diagnoses were heterogeneous, we grouped them into three categories which were defined as: i) non-oncological diseases, ii) lymphoid malignancies and iii) myeloid malignancies. With regards to radiotherapy, few other treatments besides TBI or TAI were administered to irradiated patients. Radiotherapy did not involve long bones in any of these cases, thus only TBI or TAI were considered possible risk factors.

Multivariate analysis was then performed, and only variables that proved to be of statistical significance or borderline significance in univariate analysis were included in the model, which had a p value ≤0.07 as the cut-off. The model showing the best fit was based on backward stepwise selection procedures, and each variable was removed if it did not contribute significantly. In the final model, a p value <0.05 was considered statistically significant, and all p values were based upon two-tailed tests. Statistical analyses were performed using SPSS for Windows (SPSS Inc, Chicago, Illinois, USA).

Results and Discussion

Eight (53%) of the 15 AIEOP-SCT centers agreed to participate in this study. Altogether, they had performed 1,091 allogeneic SCT in as many children, and reported that 43 (3.9%) patients had developed osteonecrosis. Osteonecrosis was diagnosed at a median age of 14.4 years (mean 14.4±3.9) and at a median interval of 10.8 months (mean 15.6±22) after SCT. We were able to identify three controls for each case, i.e., a total of 129 transplanted subjects without osteonecrosis. The main characteristics of the two groups are reported in Table 1. Univariate analysis (Table 2) showed no significant effects with regards to donor type, continuous steroid use prior to SCT, or acute GvHD, while only a borderline increased risk of osteonecrosis was observed for females and for patients with a diagnosis of acute lymphocytic leukemia or non Hodgkin's lymphoma. The factors that were found to be significantly associated with an increased risk of osteonecrosis were: having received SCT at an older age (13.1±3.2 vs. 8.1±4.2 years; OR 1.39; 95% CI 1.24-1.57), or having received radiotherapy as part of the conditioning regimen (OR 5.73; 95% CI 2.38-13.83). Among patients treated with radiotherapy, those who received TBI in three fractions had a 4.74 fold increased risk of osteonecrosis (95% CI 1.78-12.63) as compared to those treated with six fractions. Other factors that were found to be related to an increased risk of osteonecrosis were longer duration of acute GvHD (OR 1.01; 95% CI 1.002-1.027), and of

Table 1. Characteristics of patients with Osteonecrosis and matched controls.

Characteristics of patients	*Cases N =43*	*Controls N=129*
Age at SCT, years	13.1±3.2	8.06±4.2
Gender, n (%)		
Male	18 (42)	75 (58)
Female	25 (58)	54 (42)
Underlying diseases, n (%)		
Acute lymphocytic leukemia	22 (51)	52 (40)
Acute myeloid leukemia	8 (19)	24 (19)
Severe aplastic anemia	5 (12)	15 (12)
Non-Hodgkin's lymphoma	5 (12)	2 (2)
Chronic myeloid leukemia	2 (5)	15 (12)
Inborn errors	1 (2)	9 (7)
Myelodysplastic syndrome	–	7 (5)
Histiocytosis	–	5 (4)
Donor type, n (%)		
Related donor	26 (61)	88 (68)
Unrelated donor	17 (39)	41 (32)
Radiotherapy, n (%)		
No	7 (16)	68 (53)
Yes	36 (84)	61 (47)
Type of TBI, n (%)		
Six fractions (1200 cGy)	18 (50)	57 (79)
Three fractions (990 cGy)	18 (50)	13 (21)
Grade of acute GvHD, n (%)		
Absent	6 (14)	35 (27)
1–2	28 (65)	70 (54
3–4	9 (21)	24 (19)
Grade of chronic GvHD, n (%)		
Absent	13 (31)	84 (68)
Limited	13 (31)	25 (20)
Extensive	16 (38)	14 (11)

Table 2. Clinical risk factors for osteonecrosis following allogeneic SCT: univariate analysis.

Risk factors	*No cases N=43*	*No controls N=129*	*OR*	*95% CI*	*p*
Age at SCT, years	13.1±3.2	8.1±4.2	1.39	(1.24-1.57)	0.0001
Gender					
Male	18	75	ref		
Female	25	54	1.93	(0.96-3.88)	0.06
Underlying disease					
SAA, MDS, inborn errors, histiocytosis	6	36	ref		
AML, CML	10	39	1.54	(0.51-4.66)	0.053
ALL, NHL	27	54	2.99	(1.13-7.99)	
Donor type					
Related donor	26	88	ref		
Unrelated donor	17	41	1.4	(0.69-2.87)	0.35
Radiotherapy					
No	7	68	ref		
Yes	36	61	5.73	(2.38-13.83)	0.0001
Type of Radiotherapy					
TBI six fractions (1200 cGy)	18	48	ref		
TBI three fractions (990 cGy)	16	9	4.74	(1.78-12.63)	0.008
TAI single dose (600 cGy)	2	4	1.33	(0.22-7.92)	
Acute GvHD					
No	6	35	ref		
Yes	37	94	2.29	(0.89-5.91)	0.08
Grade of acute GvHD					
Absent	6	35	ref		
1-2	28	70	2.33	(0.88-6.15)	0.225
3-4	9	24	2.19	(0.69-6.95)	
Duration of acute GvHD, (days)	51.5±32	37.5±30	1.014	(1.002-1.027)	0.02
Chronic GvHD					
No	13	84	ref		
Yes	30	45	4.31	(2.05-9.07)	0.0001
Grade of chronic GvHD					
Absent	13	84	ref		
Limited	13	25	3.36	(1.38-8.17)	0.0001
Extensive	16	14	7.38	(2.93-18.62)	
Duration of chronic GvHD, (months)	32.1±36	18.4±19	1.02	(1.000-1.037)	0.05
Continuous steroids before SCT					
No	17	49	Ref		
Yes	26	80	0.94	(0.46-1.90)	0.86
Continuous steroids after SCT					
No	1	26	ref		
Yes	42	103	10.53	(1.39-79)	0.02
Duration of steroids after SCT, (months)	7.9±5.5	5.4±4	1.118	(1.034-1.209)	0.005
Cumulative dose of MPD (mg/Kg)	124.8±96	109.7±99	1.001	(0.998-1.005)	0.41

SAA: severe aplastic anemia; MDS: myelodysplastic syndrome; ALL: acute lymphocytic leukemia; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; NHL: non Hodgkin's lymphoma; TBI: total body irradiation; TAI: thoracoabdominal irradiation; GvHD: graft-versus-host disease; MPD: methylprednisolone; ref.: reference.

Table 3. Risk factors for osteonecrosis: multivariate logistic regression analysis.

Risk factors	*OR*	*95% CI*	*p*
Chronic GvHD			
Absent	Ref.		0.001
Limited	1.7	(0.54-5.31)	
Extensive	9.7	(2.86-32.8)	
Radiotherapy during conditioning regimen			
No	Ref.		0.002
TBI six fractions (1200 cGy)	2.9	(0.83-10)	
TBI three fractions (990 cGy)	20.0	(3.99-100)	
TAI single dose (600 cGy)	16.7	(1.62-172)	
Age at SCT, years	1.44	(1.24-1.68)	<0.0001

Ref.: reference; GvHD: graft-versus-host disease; TBI: total body irradiation; TAI: thoracoabdominal irradiation; cGy: centigrays.

chronic GvHD (OR 4.31; 95% CI 2.05-9.07), with a strong correlation with the grade of chronic GvHD (Table 2). Comparison between limited and extensive chronic GvHD alone showed that children with extensive disease had a 2.20 fold increased risk of osteonecrosis (95% CI 0.82-5.86), but the difference was not statistically significant (p=0.12). Finally, the continuous use of steroids after SCT was found to be a significant risk factor for osteonecrosis (OR 10.53; 95% CI 1.39-79; p=0.02), as was their use for longer periods of time (7.9±5.5 months among cases vs. 5.4±4.0 months among controls; p=0.005). However, no significant differences were found when the cumulative dose of methylprednisone administered after SCT was taken into consideration (124.8±96 mg/kg among cases, vs. 109.7±99 mg/kg among controls; p=0.41).

Multivariate logistic regression analysis (Table 3) showed that the only factors that were significantly associated with an increased risk of osteonecrosis were; extensive chronic GvHD (OR=9.7; 95% CI 2.87-32.8, p=0.001), TBI administered in three fractions (OR=19.2, 95% CI 4.16- 89; p=0.001), and older age at transplantation (OR=1.45, 95% CI 1.25-1.68; p=0.0001).

To our knowledge, this is the first report that specifically focuses on the analysis of risk factors for osteonecrosis after allogeneic SCT in children. The 3.9% incidence we observed is within the 0.3%-9% range reported in the only two studies which also included pediatric patients.[5,6] It must be pointed out, however, that in our series, as in others, the incidence may be slightly underestimated since heterogeneous criteria for diagnosis were adopted. In these studies, children always had a lower incidence of osteonecrosis than had adults, and the risk increased with age at SCT in our study. We believe that this could be explained by the fact that a growing bone has a greater ability to remodel after any injury that may occur during treatment.

The possible role of steroids as a causative factor of osteonecrosis was speculated in only few other reports involving leukemic children treated with front line therapy. However, these studies reported a lower (between 1.1% and 1.8%) incidence of osteonecrosis.[11,12] Fink *et al.*, in a 1:1 case-control study on adult recipients of allogeneic or autologous SCT, observed an increased risk of

osteonecrosis due to steroids and TBI, but did not demonstrate any effect related to the duration of steroid use.[3] In another study on allogeneic SCT recipients, Sociè *et al.*[5] did not directly analyze the effect of steroids, but, they did hypothesize an indirect association between GvHD and steroid treatment since patients without GvHD in their series had a lower risk of osteonecrosis. In our study, continuative and longer use of steroids after SCT were found to be associated with the risk of osteonecrosis, but their effects disappeared in multivariate analysis; moreover, no effects related to cumulative dose were observed. The role played by steroid administration prior to SCT does not seem to be important. In fact, univariate analysis showed that, neither steroid administrated prior to SCT nor a primary diagnosis of acute lymphoblastic leukemia or non-Hodgkin's disease requiring prolonged steroid treatment were significant for the risk of osteonecrosis.

We found that TBI (in particular when given in 330 cGy fractions for 3 days), chronic GvHD, and older age at SCT are the most important determinants of osteonecrosis after SCT. We hypothesize that osteonecrosis is mainly due to vascular damage secondary to the synergistic effect of cGvHD and TBI. In fact, vascular damage may be the consequence of early and late toxicity of radiotherapy, which is known to induce both a reduction in the number of osteoblasts and micro-vascular changes due to sub-intimal fibrosis and thickening of the median layer of small vessels.[13] Animal models have also demonstrated that vascular injury with intimal hyperplasia and adventitial fibrosis is the primary event of chronic GvHD, which will eventually lead to narrowing of the arterial lumina, and tissue ischemia.[14] We hypothesize that, as suggested by Sociè *et al.*, steroids per se do not significantly increase the risk of osteonecrosis, but that they are a surrogate marker of GvHD. The fact that patients treated with six fractions of TBI (total dose 1200 cGy) had a lower risk than those treated with three fractions (total dose 990 cGy) is not surprising. It is well known that administering radiotherapy twice a day (200 cGy per fraction) has a higher killing effect on cancer cells and lower biological toxicity on the surrounding tissues as compared to radiotherapy given in a single dose (330 cGy). This strategy of dividing the daily dose therefore reduces the risk of early and late complications secondary to radiotherapy. We cannot comment on the effect of single dose administration of TAI due to the small number of children treated with this technique.

In conclusion, changes in blood fluid after TBI and/or chronic GvHD-related vasculitis in a bone that has likely become osteoporotic because of prolonged steroid treatment, as well as a history of chronic disease and irradiation all represent predisposing factors for the development of osteonecrosis.

MF, RH contributed to the design, conduction and analysis of the study and wrote the paper; MGC, SC, with RR contributed to statistical analysis, data entry, and identification of controls in the AIEOP registry; EL, CM, CF, AI, SS, SB, and GD contributed to study planing, case ascertainement, data collection, and interpretation of results. Preliminary data of this study were presented at the EBMT meeting in Barcellona. This work was supported in part by Ricerca Finalizzata Ministeriale 2002, by the CARIGE Foundation, and by the San Paolo Company. Manuscript received February 22, 2006. Accepted May 31, 2006.

References

1. Atkinson K, Cohen M, Biggs J. Avascular necrosis of the femoral head secondary to corticosteroid therapy for graft-versus-host-disease after marrow transplantation: effective therapy with hip arthroplasty. Bone Marrow Transpl 1987;2:421-6.
2. Tauchmanovà L, De Rosa G, Serio B, Fazioli F, Mainolfi C, Lombardi G, et al. Avascular necrosis in long-term survivors after allogeneic or autologous stem cell transplantation. Cancer 2003; 97:2453-61.
3. Fink JC, Leisenring WM, Sullivan KM, Sherrard DJ, Weiss NS. Avascular necrosis following bone marrow transplantation: a case-control study. Bone 1998; 22:67-71.
4. Socié G, Sélimi F, Sedel L, Frija J, Devergie A, Esperou Bourdeau H, et al. Avascular necrosis of bone after allogeneic bone marrow transplantation: clinical factors, incidence and risk factors. Br J Haematol 1994;86:624-8.
5. Socié G, Cahn JY, Carmelo J, Vernant JP, Jouet JP, Ifrah N, et al. Avascular necrosis of bone after allogeneic marrow transplantation: analysis of risk factors for 4388 patients by the Society Françoise de Greffe de Moelle (SFGM). Br J Haematol 1997;97:865-70.
6. Enright H, Haake R, Weisdorf D. Avascular necrosis of bone: a common serious complication of allogeneic bone marrow transplantation. Am J Med 1990;89:733-8.
7. Balduzzi A, Gooley T, Anasetti C, Sanders JE, Martin PJ, Petersdorf FW, et al. Unrelated donor marrow transplantation in children. Blood 1995;86:3247-56.
8. Torii Y, Hasegawa Y, Kubo T, Kodera Y, Minami S, Morishita J, et al. Osteonecrosis of the femoral head after allogeneic bone marrow transplantation. Clin Orthop 2001;382:124-32.
9. Schulte CM, Beelen DW. Avascular osteonecrosis after allogeneic hematopoietic stem – cell transplantation: diagnosis and gender matter. Transplantation 2004;78:1055-63.
10. Mattano L. The skeletal remains: porosis and necrosis of bone in the marrow transplantation setting. Pediatr Transplant 2003;7:71-5.
11. Aricò M, Boccalatte MF, Silvestri D. Barisone E, Messina C, Chiesa R, et al. Osteonecrosis: an emerging complication of intensive chemotherapy for childhood acute lymphoblastic leukemia. Haematologica 2003;8:747-53.
12. Burger B, Beier R, Zimmermann M, Beck JD, Reiter A, Shrappe M. Osteonecrosis: a treatment related toxicity in childhood acute lymphoblastic leukemia (ALL)-Experience from trial ALL-BFM 95. Pediatr Blood Cancer 2005;44:220-5.
13. Hopewell JW. Radiation-therapy effects on bone density. Med Pediatr Oncol 2003;41:208-11.
14. Shulman HM, Sale GE. Pathology of acute and chronic cutaneous GVHD. In: Sale GE and Shulman HM, editors. The Pathology of Bone Marrow Transplantation. USA: Masson Publishing Inc. 1984. p. 68-76.