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ARTICLE

Occurrence of long-term effects after hematopoietic stem cell transplantation in children affected by acute leukemia receiving either busulfan or total body irradiation: results of an AIEOP (Associazione Italiana Ematologia Oncologia Pediatrica) retrospective study

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Abstract

Patients given allogeneic hematopoietic stem cell transplantation (alloHSCT) present an increased incidence of long-term toxicities that can be attributed to the preparative regimen. We retrospectively analyzed in a population of 670 children receiving allo-HSCT for acute leukemia the occurrence of different late effects in function of the choice made between total body irradiation (TBI) and busulfan, as part of the preparative regimen. In univariable analysis, we found that patients treated with TBI developed cataract in 24% of the cases compared with 4% in patients treated with BU ($p = 0.0001$) and that the incidence of secondary malignant neoplasia (SMN) was higher in patients treated with TBI (18%) as compared with those prepared to the allograft with a Bu-based regimen (0%) ($p = 0.019$). Conditioning regimen did not show a statistically significant correlation with the occurrence of all the other investigated late effects. In multivariable analysis, TBI remained associated with the occurrence of cataracts (Relative Risk: 0.33 $p = 0.012$) and secondary malignancies (Relative Risk $3.96 \times 10e{-}6$ $p < 0.001$); however, other variables, as GvHD and disease type, were also correlated with these long-term sequels, indicating that in our study population the preparative regimen is not the only factor influencing the incidence of these complications.

Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is a consolidated therapy for many oncological diseases and for some non-malignant disorders affecting the hematopoietic and the immune system [1]. Although the results of alloHSCT have improved over years, patients receiving this procedure during their childhood and

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becoming long-term survivors still have a shorter life expectancy compared with both sex- and age-matched healthy subjects and patients treated with conventional chemotherapy, mainly because of an increased incidence of long-term effects [2, 3]. Moreover, it has been reported that the occurrence of long-term effects after alloHSCT, even when it does not cause the patient's premature death, could still remarkably affect the quality of life of pediatric patients [4]. Every system and every organ can be involved; in particular, target of long-term toxicities of alloHSCT may be the cardiovascular and respiratory system, endocrine function (including the reproductive system), the sensory (especially the eye and the ear), bone, metabolic function, renal function, liver, gastrointestinal tract and the skin [5]. The increased incidence of late effects after alloHSCT has often been attributed, at least in part, to a late toxicity of the preparative regimen [6], which, in the pediatric population affected by acute leukemias, is usually based on the administration of either total body irradiation (TBI) or busulfan (Bu). In patients under the age of 3, TBI is usually omitted because of its very high rate of long-term toxicity in this group of patients [7, 8]. In older children, the use of TBI or Bu, as backbone for preparative regimen, has been compared in both prospective and retrospective studies aimed at identifying differences in terms of efficacy and early toxicity for both acute lymphoblastic leukemia (ALL) [9, 10] and acute myelogenous leukemia (AML) [11–13]. These studies showed that, especially for ALL, the inclusion of TBI in the conditioning is associated with reduction of relapse rate [9, 10].

Data available on long-term toxicity after TBI or Bu exposure are limited and contradictory: some authors reported the same incidence of alterations of growth in children receiving either TBI or Bu during the conditioning regimen [14, 15], while others reported an increased risk of endocrine complications [16–18], cataract [18] and secondary malignant neoplasia [19] in patients who had received TBI. More recently, a retrospective analysis performed on a large population of childhood cancer survivors given HSCT showed a reduced incidence of late complications after Bu. In particular, that study found a correlation between the use of TBI and growth impairment, cataract, and iron overload; a correlation between Bu administration and the overweight and alopecia was found, as well [20].

In consideration of the particular importance that late effects has in the pediatric population, and of the role that TBI has in the disease control in pediatric hematological malignancies [21], in this multicenter study, we investigated the impact of the use of either TBI or Bu in the conditioning regimen on the occurrence of late effects.

Subjects and methods

Patients

The study included all consecutive patients who underwent first alloHSCT for acute leukemia between January 1st 2000 and December 31st 2012 in one of the 20 pediatric Italian transplant Centers affiliated to AEIOP (Associazione Italiana Ematologia ed Oncologia Pediatrica). To better identify long-term effects, analyses were performed for all patients when they reached a follow up of at least 5 years after the allograft. Data were retrieved from the AIEOP-HSCT Registry where every AIEOP Center reports information on patient, donor and transplant characteristics, as well as on outcome, according to policies approved by the local Ethic Committee and after obtaining informed consent from parents or legal guardians.

Inclusion criteria were: (i) age comprised between 3 and 18 years at the time of alloHSCT; (ii) diagnosis of either ALL or AML; (iii) first myeloablative alloHSCT from HLA-identical sibling donor or HLA-matched unrelated donor or HLA-haploidentical donor; (iv) bone marrow, peripheral blood stem cells and cord blood as stem cell source (v) conditioning regimen including either intravenous Bu (cumulative dose from 16 mg/kg to 12.8 mg/kg) or oral Bu (cumulative dose 16 mg/kg) or TBI (fractionated total dose equal to or greater than 990 cGy); and (vi) complete morphological remission before starting the preparative regimen and (vii) survival with disease remission longer than one year after the transplantation.

Exclusion criteria were history of previous allogeneic HSCT, diagnosis of ALL or AML in the context of Down syndrome or of other diseases characterized by chromosomal instability syndromes (i.e., AML in Fanconi Anemia), and diagnosis of AML or ALL secondary to previous exposure to chemotherapy or radiation. Patients underwent clinical and hematological assessments both before and after transplantation according to each Center's policy.

Definitions and endpoints

The aim of this study was to evaluate the impact of TBI and Bu (both oral and i.v. formulation) on the occurrence of long-term sequels in a large population of pediatric patients undergoing alloHSCT for acute leukemia.

As long-term effects, we considered occurrence of growth impairment, alteration of gonadal function, alteration of thyroid function, cataract, occurrence of secondary malignant neoplasia and alteration of pulmonary function in subjects surviving for more than one year after alloHSCT.

If asymptomatic, all patients were screened for the occurrence of long-term effects according to previously published guidelines [22, 23] and when a toxicity was identified a

patient-specific diagnostic work up was established. Apart from scheduled follow-up visits, patients and their families were also encouraged to contact the Transplant Center at the onset of new symptoms or worsening of already known health problems, in order to identify toxicities in their early stage.

All patients had evaluations including height, weight, body mass index (BMI), and Tanner staging every 6 months and if abnormalities were noted they were referred to endocrinologist to discuss a specific diagnostic work up, including growth hormone dosing.

Growth impairment was defined as either failure to reach normal height and weight for age according to the patient's age and genetic target or as a growth rate under the 3rd percentile.

Alteration of gonadal function in male patients was defined as either delayed puberty, or as precocious puberty, or as reduction of age-adjusted testosterone blood levels associated with high levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), or as alteration of number, shape or motility of spermatozoa in semen.

Alteration of gonadal function in female patients was defined as either delayed puberty, precocious puberty, secondary amenorrhea requiring hormonal replacement therapy, or reduction of age-adjusted estradiol blood levels with high levels of FSH and LH.

Alteration of thyroid function was defined as elevation of thyroid-stimulating hormone, associated or not with decreased levels of free thyroxine or triiodothyronine in repeated controls, requiring specific therapy, or as thyroiditis resulting in hypothyroidism.

Patients were screened for cataract during specific screening exams conducted by an ophthalmologist at least every 1–3 years after the transplantation.

Secondary neoplasia screening was performed by physicians with experience in the diagnosis and the in treatment of long-term effects after HSCT during scheduled follow-up visits or when clinically indicated.

Patients were tested for pulmonary function by spirometry at one-year post-transplant or once age-appropriate. In case of inability to perform standard pulmonary function testing and when a pulmonary toxicity was suspected an alternative testing method was discussed with pulmonology in every single case. Re-evaluation of pulmonary function was per clinician discretion and it was guided by initial findings.

Transplant-related mortality (TRM) is defined as the probability of dying without a previous relapse occurrence. If the patient either experienced relapse or is still alive at the end of the study time, data are censored at the relapse date or at last follow-up date respectively.

Acute GvHD was diagnosed and staged according to previously reported criteria [24]. For chronic GvHD considering that many data had been collected before current staging system was adopted [25], previous staging system was used [26].

Overall survival (OS) is defined as the probability of survival irrespective of the disease state at any point in time. If, at the end of the study time, the patient is still alive data are censored at the last follow-up date.

Statistical analysis

To assess the influence of TBI and Bu administration on occurrence of late effects, a two-tailed Fisher Test was performed. In order to identify a correlation between the occurrence of each specific late effect and exposure to either Bu or TBI, we first performed univariable analysis including also the following other variables: patient gender, age at HSCT, disease, donor type, disease status at HSCT, aGvHD, cGvHD and the length of the follow-up. The occurrence of each late effect was calculated as cumulative incidence (CI) to adjust the analysis for competing risks: both leukemia relapse and death were considered competing risks. If the patient either experienced relapse or is still alive at the end of the study time, data are censored at the relapse date or at last follow-up date respectively. TRM was also calculated as a cumulative incidence (CI) considering disease recurrence as competing risk. The differences in terms of CI were compared using Gray's test. To perform multivariable analyses, we selected variables reaching statistical significance in the univariable analyses. Multivariable analysis was performed using logistic regression. OS was calculated according to the Kaplan–Meier method and the significance between the observed differences was established by the log-rank test. In all the analyses, a p value less than 0.05 was considered statistically significant. All the statistical analyses were performed using NCSS (Hintze, 2001; NCSS PASS, Number Crunched Statistical System, Kaysville, UT, USA) and R 2.5.0 software packages.

Results

The study included 670 patients (415 males and 255 females) with a median age of 9 years (range 3–18) at time of alloHSCT and with a median follow-up of 7 years (range 5–16) after HSCT.

Analysis used May 1st 2017 as reference date; 473 patients (70%) had received TBI, while 197 (30%) Bu. Patient and transplant characteristics are summarized in Table 1.

Median duration of follow up was of 7 years (range: 3–16) in the TBI group and 6 years (range: 3–16) in the Bu group.

Overall, 38% of patients ($n = 254$) presented one single late effect and 14% of patients ($n = 93$) presented more than one late effect. The incidence of at least one late complication was higher in TBI-treated patients than in Bu patients (48% vs 28%, $p = 0.001$). Similarly, the occurrence of more

Table 1 Patients and transplants characteristics.

	TBI $n = 473$	Bu $n = 197$	p
Gender			
Male	62% (294)	61% (121)	0.92
Female	38% (179)	39% (76)	
Age			
3–5 years	11% (53)	17% (33)	0.24
5–10 years	44% (208)	34% (66)	
10–15 years	30% (143)	38% (74)	
15–18 years	15% (69)	11% (24)	
Disease			
ALL	95% (450)	24% (47)	<0.0001
AML	5% (23)	76% (150)	
Phase			
CR1	36% (170)	74% (145)	<0.0001
CR2	58% (273)	25% (49)	
Others	6% (30)	1% (3)	
Donor			
MFD	38% (179)	51% (101)	0.04
UD	50% (239)	42% (82)	
PMFD	12% (55)	7% (14)	
HSC source			
BM	75% (353)	79% (155)	0.51
PBSC	16% (74)	14% (27)	
CB	9% (46)	7% (15)	
Acute GvHD			
Absent	33% (155)	39% (76)	0.21
Grade 1	27% (127)	22% (44)	
Grade 2	32% (151)	28% (55)	
Grade 3	7% (34)	8% (16)	
Grade 4	1% (6)	3% (6)	
Chronic GvHD			
Absent	73% (346)	72% (141)	0.25
Limited	15% (70)	19% (38)	
Extensive	12% (56)	9% (18)	

ALL acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *CR* complete hematological remission, *MFD* matched family donor, *UD* unrelated donor, *PMFD* partially matched familiar donor, *BM* bone marrow, *PBSC* peripheral blood hematopoietic stem cells, *CB* cord blood.

than two late effects was more frequent in the TBI group (16% vs 7%, $p = 0.006$).

At 5 years TRM of TBI-treated and of Bu-treated patients were 3% (95% CI: 1–5) and 3% (95% CI: 1–6), respectively ($p = 0.97$). At 9 years OS of TBI-treated and of Bu-treated patients were 92% (95% CI: 89–95) and 92% (95% CI: 87–96), respectively ($p = 0.99$).

Growth alteration

Among patients given TBI, 29% (95% CI: 20–43) had growth retardation compared with 18% (95% CI: 10–31) in Bu-treated patients; however, this difference was not statistically significant ($p = 0.45$) (Table 2 and Fig. 1). Patient age, donor type and aGvHD in our population showed a significant correlation with the occurrence of alteration of the growth (Table 3), while gender, disease type, disease status at alloHSCT, cGvHD and length of the follow up did not show any correlation with this late effect. Considering the seriousness of growth alteration, we didn't observe any difference between TBI and Bu group: patients with growth impairment needing Growth Hormone were 4 and 3% in the two groups respectively.

Alteration of gonadal function

Patients treated with TBI had alteration of gonadal function in 41% of the cases (95% CI: 28–61), while patients treated with Bu in 20% (95% CI: 15–30, $p = 0.14$) (Table 2 and Fig. 1). Among the other variables investigated, patient age and gender were found to be correlated with the occurrence of alteration of gonadal function (Table 3), while disease type, donor type, disease status at alloHSCT, both acute and chronic GvHD and length of the follow up did not have any significant influence.

Alteration of thyroid function

Patients treated with TBI showed alteration of thyroid function in 18% of the cases (95% CI: 13–26), compared with 12% (95% CI: 7–20, $p = 0.46$) in patients treated with Bu (Table 2 and Fig. 1).

Moreover, patients with a longer follow up were more likely to develop alteration of the thyroid function; none of the other variables investigated showed a correlation with the occurrence of these abnormalities in our study population.

Cataract

We found a statistically significant correlation between the conditioning regimen and the occurrence of cataract: patients treated with TBI developed cataract in 24% of the cases (95% CI: 16–34), compared with 4% (95% CI: 2–10, $p = 0.0001$) in patients treated with Bu (Table 2 and Fig. 1). Also, disease type and aGvHD showed a statistically significant impact on the occurrence of cataract (Table 3). Patient age and gender, donor type, disease status at alloHSCT, chronic GvHD and length of the follow up did not correlate with the occurrence of this late effect. In multivariable analysis, we found that patients treated with Bu showed a reduced risk of developing cataract compared with patients undergone to TBI (Relative Risk: 0.33 95% CI: 0.14–0.78 $p = 0.012$) but also that patients who did experience acute GvHD were more likely to develop cataract as compared with patient who did not (Relative Risk grade 2 aGvHD 3.5 95% CI: 1.62–7.75 $p = 0.0015$; Relative Risk grade 3 aGvHD 7.29 95% CI: 3.06–17.36 $p <$

Table 2 Univariable analysis about the role of total body irradiation or busulfan in determining late effects.

Variable	*n*	Events	Cumulative incidence	95% CI	Grey's test
Growth impairment					
TBI	425	44	29%	20–43	0.45
Bu	189	13	18%	10–31	
Alteration of gonadal function					
TBI	449	97	41%	28–61	0.46
Bu	191	30	20%	15–30	
Alteration of thyroid function					
TBI	450	54	18%	13–26	0.46
Bu	193	18	12%	8–20	
Cataract					
TBI	448	65	24%	16–34	<0.0001
Bu	191	6	4%	2–10	
Secondary malignant neoplasm					
TBI	445	25	18%	9–38	0.019
Bu	192	0	0	–	
Alteration of pulmonary function					
TBI	444	25	6%	4–9	0.3
Bu	190	14	8%	4–13	

TBI total body irradiation, *Bu* busulfan iv or oral, *95% CI* interval of confidence at 95%.

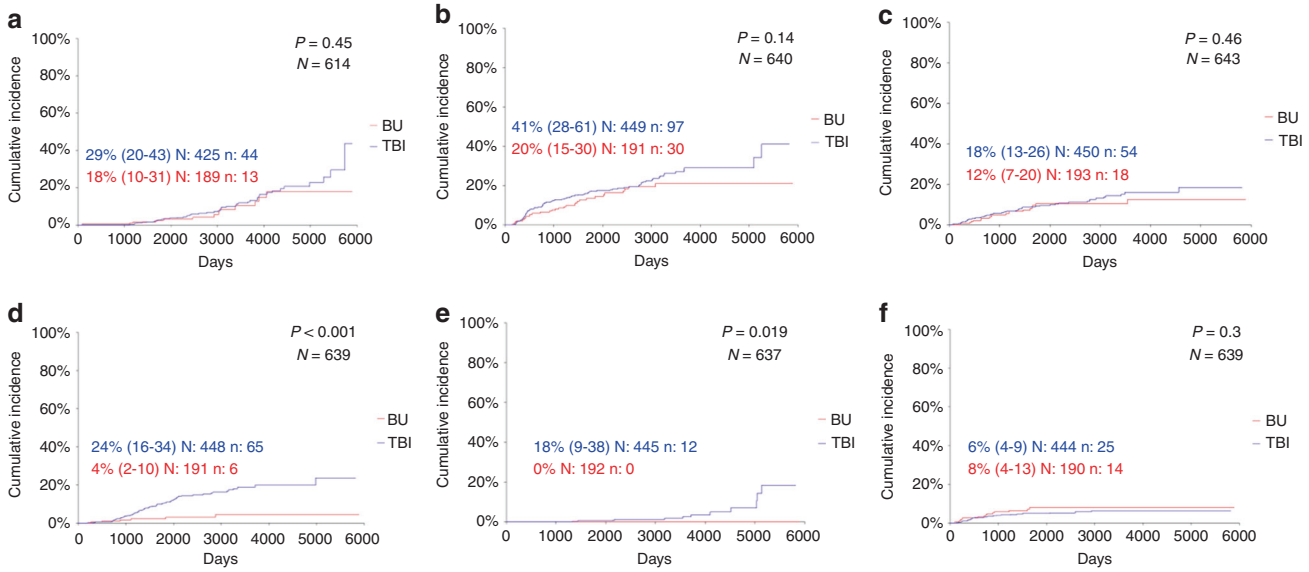

Fig. 1 Cumulative Incidence of long-term effects. Cumulative incidence of (**a**) growth alteration, (**b**) alteration of the gonadal function (**c**) alteration of the thyroid function (**d**) cataract (**e**) secondary malignant neoplasia and (**f**) alteration of the pulmonary function according to the conditioning regimen. TBI total body irradiation BU busulfan iv or oral, 95% CI interval of confidence at 95%, n events.

0.001; Relative Risk grade 4 aGvHD 13.75 95% CI: 3.26–57.9 $p < 0.001$) (Table 4). We did not find a correlation between disease type and development of cataract by multivariable analysis.

Secondary malignancies

The incidence of secondary malignant neoplasia (SMN) was higher in patients treated with TBI (18% 95% CI: 9–38) as compared with those prepared to the allograft with a Bu-based regimen (0%) and this difference was statistically significant ($p = 0.019$) (Table 2 and Fig. 1). SNM included papillary thyroid carcinomas ($n = 3$), malignant melanomas ($n = 2$), renal carcinoma ($n = 1$), a grade IV glioma ($n = 1$), undifferentiated sarcoma ($n = 2$), neurofibroma ($n = 1$), hepatocellular carcinoma ($n = 1$) and secondary acute myelogenous leukemia ($n = 1$). In univariable analysis, disease type was correlated with

Table 3 Univariable analysis about the role of sex, age, disease type, donor type, disease status at the transplant, aGvHD, cGvHD and follow up length in determining late effects: variables reaching the statistical significance.

	Variable	*n*	Events	Cumulative incidence	95% CI	Grey test
Growth impairment	Age					
	3–5 years	86	12	30%	18–52	0.001
	5–10 years	269	35	65%	50–76	
	10–15 years	204	12	19%	8–45	
	15–18 years	90	0	0	–	
	Donor					
	MFD	269	27	17%	9–31	0.012
	MUD	306	41	*33%*	23–47	
	PMFD	67	3	12%	4–33	
	aGvHD					
	Absent	201	17	24%	13–44	0.002
	Grade 1	161	16	28%	15–51	
	Grade 2	197	17	20%	12–32	
	Grade 3	50	4	22%	9–57	
	Grade 4	12	3	42%	16–100	
Gonadal alteration	Sex					
	Male	400	39	20%	10–39	<0.001
	Female	248	33	52%	41–62	
	Age					
	3–5 years	86	12	18%	8–41	<0.001
	5–10 years	269	35	32%	23–43	
	10–15 years	214	20	30%	24–38	
	15–18 years	92	5	32%	24–44	
Thyroid function	Follow-up length					
	<7 years	342	21	6%	4–10	0.01
	>7 years	291	51	18%	14–24	
Cataract	Disease					
	ALL	471	64	22%	15–33	<0.001
	AML	170	7	5%	2–11	
	aGvHD					
	Absent	223	9	5%	2–10	<0.001
	Grade 1	166	15	20%	9–45	
	Grade 2	201	30	22%	16–32	
	Grade 3	50	14	37%	24–58	
	Grade 4	12	3	25%	9–67	
SMN	Disease					
	ALL	467	12	17%	7–35	0.02
	AML	170	0	0	–	
Alteration of pulmonary function	cGvHD					
	Absent	400	13	4%	2–7	0.0011
	Limited	104	9	9%	5–16	
	Extensive	72	13	19%	11–31	

MFD matched family donor, *UD* unrelated donor, *PMFD* partially matched familiar donor, *ALL* acute lymphoblastic leukemia, *AML* acute myelogenous leukemia.

Table 4 Multivariable analysis.

	Variable	Relative risk	95% CI	*p* value
Cataract	*Conditioning*[a]	0.33	0.14–0.78	0.001
	Grade II aGVHD[b]	3.5	1.62–7.75	0.0015
	Grade III aGVHD[b]	7.29	3.06–17.36	<0.001
	Grade IV aGvHD[b]	13.75	3.26–57.9	<0.001
SMN	*Conditioning*[a]	3.96×10^{-6}	1.68×10^{-6}–9.34×10^{-6}	<0.001
	Disease[c]	2.13×10^{-6}	1×10^{-6}–9.34×10^{-6}	<0.001

SNM secondary malignant neoplasia.

[a]Compared with total body irradiation as reference.

[b]Compared with no GvHD-Grade I aGvHD.

[c]Compared with acute lymphoblastic leukemia.

SMN incidence, as well; indeed, patients affected by ALL developed SMN in 17% of cases (95% CI: 7–35), while no case of SNM was observed in patients affected by AML ($p = 0.02$) (Table 3).

Univariable analysis did not show a statistically significant correlation between patient's age and sex, donor type, disease status at alloHSCT, aGvHD, cGvHD and length of the follow up and the occurrence of SMN in our study population. In multivariable analysis, we observed that both the conditioning regimen and disease type were related to the occurrence of SMN: patients affected by ALL developed more frequently SNM compared with patients affected by AML (Relative Risk $2.13 \times 10e{-}6$ 95% CI: $1 \times 10e{-}6$–$4.45 \times 10e{-}6$ $p < 0.001$) and patients treated with TBI more frequently developed SNM compared with patients given Bu during the preparation to the allograft (Relative Risk $3.96 \times 10e{-}6$ 95% CI: $1.68 \times 10e{-}6$–$9.34 \times 10e{-}6$ $p < 0.001$) (Table 4) In order to evaluate the impact of SNM on survival in our study population, we evaluated OS in patients developing SNM and in patients who did not: OS at 9 years was 80% (95% CI: 55–100) and 92% (95% CI: 90–95) ($p = 0.3$), respectively.

Pulmonary toxicity

Considering lung toxicity, we observed a similar incidence of pulmonary late effects in patients treated with either TBI or with Bu: 6% (95% CI: 4–9) and 8% (95% CI: 4–13) respectively ($p = 0.3$) (Table 2 and Fig. 1). Among the other variables investigated in the univariable analysis only the occurrence of chronic GvHD was shown to correlate with lung function impairment in a statistically significant manner (Table 3). Patient age and sex, disease type, donor type, disease status aGvHD and follow-up length did not show any correlation with the occurrence of alterations of the pulmonary function.

Discussion

AlloHSCT is able to offer to many children affected by several both malignant and non-malignant diseases the best opportunities to be cured; however, the risk of developing long-term toxicity is still a matter of concern for pediatric hematologists and oncologists to be carefully considered in planning the therapeutic strategy for their patients [4]. Together with monitoring strategies aimed at timely diagnosing the occurrence of late effects [22], one of the variables more suitable for intervention is the choice of the preparative regimen. Since the first alloHSCTs performed in the late Seventies, TBI emerged as the treatment modality able to meet the requirements of the ideal conditioning agent, because its ability to abrogate original recipient's hematopoiesis, to induce the state of immunosuppression essential for a stable donor's hematopoietic stem cells engraftment and to kill residual neoplastic cells [27]. However, TBI has since been reported as one of the main causes of long-term toxicity [28].

The most used alternative to TBI, as backbone for the preparative regimen in pediatric patients, is represented by Bu. Studies comparing TBI with chemo-only preparative regimen based on Bu and specifically focusing on the occurrence of late effects in the pediatric population are few, usually based on small series of data and sometimes their results are contradictory. Wingard et al. reported the same rate of growth impairment in 47 pediatric patients affected by different diseases and treated with either TBI or Bu as part of preparative regimen [14]. Bernard et al. in 2009 described a series of 58 children undergoing alloHSCT and receiving either fractioned TBI or Bu and they concluded that preparations including Bu, even if less toxic than those based on the use of TBI, also have adverse effects on growth [15]. The largest study, investigating the differences in terms of incidence of late effects between patients treated with either TBI or Bu, was published more recently by Bernard et al. These authors concluded that late-

effects induced by TBI are more frequent and that they differ from the ones observed after exposure to Bu: TBI was correlated with an increased risk of growth impairment, cataract and iron overload, while Bu administration was associated with overweight and alopecia [20].

To increase the knowledge about the role of TBI and Bu in determining long-term effects, in this study, we retrospectively evaluated the incidence of growth impairment, alteration of gonadal function, alteration of thyroid function, cataract, SMN and alteration of pulmonary function in a large population of pediatric patients given alloHSCT for acute leukemia in the years 2000–2012. Comparing the general occurrence of long-term toxicities in patients exposed to either TBI or Bu, we observed that patients exposed to TBI are more likely to develop both a single or two or more late effects, as previously reported [20]. However, by analyzing the incidence of each single late effect, for most of them (alteration of growth, alteration of gonadal function, alteration of thyroid function and alteration of pulmonary function), it was not possible to demonstrate a statistically significant correlation with the preparative regimen, even if a trend toward a higher rate of toxicity with TBI was noticed. In the case of cataract, in univariable analysis, we observed a statistically significant correlation with the use of TBI, but also with occurrence of aGvHD and disease type. When we performed a multivariable analysis including these variables, even if it was still possible to demonstrate an increased risk of developing cataract after exposure to TBI, also the occurrence of aGvHD still remained significant, highlighting that the preparative regimen is not the only factor that can predispose to develop this complication. We confirmed that exposure to TBI increases the risk of developing SNM as previously reported [19] and in our study also disease type showed a correlation with the occurrence of this late effect: patients affected by ALL showed an increased risk as compared with patients with AML. One explanation of this latter finding could be that the majority of patients affected by ALL received alloHSCT after two previous lines of chemotherapy while most of the patients affected by AML were transplanted in first complete remission. Another explanation of increased risk to develop SMN for ALL patients could be that some of them were enrolled in first- and second-line protocols including cranio-spinal radiation therapy to prevent or treat Central Nervous System leukemia involvement before undergoing alloHSCT.

However, data about secondary malignancies should be considered with a peculiar attention because, for this late effect, median duration of the follow up of this study (7 years) may preclude the detection of some forms characterized by long latencies.

Our findings, differing from those of a previous report [20], may be interpreted in view of the fact that we chose a statistical model based on cumulative incidence to adjust the analysis for competing risks in order to have a more precise estimate of the incidence of late-effects, censoring patients not at risk of presenting this complication, either because they experienced disease relapse or because they died for transplant-related complications. Moreover, our data confirming a correlation between exposure to TBI and the occurrence of SMN after alloHSCT underline the need to identify patients at high risk of developing this complication and to carefully evaluate for them the potential benefits and risks related to the use of TBI-based preparative regimens.

In our series of patients, we did not observe a significative impact of conditioning regimen on late TRM (at 5 years 3% in both TBI and Bu group) and OS (at 9 years 92% in both TBI and Bu group) even if we observed a trend to a reduced survival for patients developing SNM (80% vs 92% $p = 0.3$) that were all reported in TBI group.

Two intrinsic limitations of our study are represented by its retrospective design and by the fact that our study populations (i.e., children given either TBI or Bu) have some base-line differences including disease type, disease status at transplantation and type of donor. These differences are in large part attributable to the inclusion in the study of patients affected by both ALL and AML, who have different indications to alloHSCT and usually are treated with different preparative regimens [29, 30] that usually are based on TBI for ALL and Bu for AML; the use of multivariable analyses, however, was intended to compensate for these differences. The retrospective design of the study unfortunately precluded us to collect some information that could have been of potential interest, as the assessments of the Quality of the Life of patients.

In conclusion, our data underline that the choice of a TBI-based preparative regimen is not the only factor able to influence the incidence of long-term effects after alloHSCT, that the type of late effects differs according to the preparative regimen administered and that specific monitoring strategies are needed. Along this direction, some groups have started to investigate if strategies based on the screening for a genetically determined susceptibility to development of long-term effects are feasible, but results are still preliminary [31]. Besides these further evolutions in the management of long-term toxicities after HSCT, nowadays it is desirable that future and ongoing prospective clinical trials, apart from evaluating the efficacy and early toxicity of preparative regimens, also consider their impact on the occurrence of late effects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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