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Gonadal Function after Busulfan Compared with Treosulfan in Children and Adolescents Undergoing Allogeneic Hematopoietic Stem Cell Transplant



Maura Faraci^{1,*}, Tamara Diesch², Myriam Labopin³, Arnaud Dalissier⁴, Arian Lankester⁵, Andrew Gennery⁶, Mikael Sundin⁷, Duygu Uckan-Cetinkaya⁸, Marc Bierings⁹, Anke M.J. Peters¹⁰, Martina Garwer¹¹, Ansgar Schulz¹², Gerard Michel¹³, Giovanna Giorgiani¹⁴, Bernd Gruhn¹⁵, Franco Locatelli¹⁶, Stefano Giardino¹, Anne Uyttebroeck¹⁷, Fanny Riolland¹⁸, Maija Itäla-Remes¹⁹, Peter Dreger²⁰, Peter J. Shaw²¹, Victoria Bordon²², Paul G. Schlegel²³, Karin Mellgren²⁴, Jose M. Moraleda²⁵, Katharine Patrick²⁶, Pascale Schneider²⁷, Charlotte Jubert²⁸, Anita Lawitschka²⁹, Nina Salooja³⁰, Grzegorz W. Basak³¹, Selim Corbacioglu³², Rafael Duarte³³, Peter Bader³⁴, on behalf of the Pediatric and Transplant Complications Working Parties of the European Society for Blood and Marrow Transplantation

¹ Istituto G. Gaslini, Hematopoietic Stem Cell Transplantation Unit- Hematology-Oncology, Genova, Italy

² University Children's Hospital Basel, Division of Pediatric Oncology/Hematology, Basel, Switzerland

³ Hôpital Saint-Antoine, Department of Hematology and Cell Therapy, Paris, France

⁴ EBMT Paris Study Office/CEREST-TC, Saint Antoine Hospital, Department of Hematology, Paris, France

⁵ Willem-Alexander Children's Hospital, Leiden University Medical Center, Department of Pediatric Stem Cell Transplantation, Leiden, Netherlands

⁶ Children's Hospital Newcastle upon Tyne, Pediatric Team Children's BMT Unit, Newcastle upon Tyne, United Kingdom

⁷ Astrid Lindgren Children's Hospital, Karolinska University Hospital, Hematology/Immunology Section, Stockholm, Sweden

⁸ Hacettepe University Faculty of Medicine, Bone Marrow Transplantation Unit, Department of Pediatrics, Ankara, Turkey

⁹ Princess Maxima Centre for Pediatric Oncology and University Children's Hospital, Utrecht, Netherlands

¹⁰ Medical Center, University of Freiburg, Center for Pediatrics, Department of Pediatric Hematology and Oncology, Freiburg, Germany

¹¹ University Hamburg-Eppendorf, Pediatric Hematology Clinic and Policlinic of Oncology, Hamburg-Eppendorf, Germany

¹² University Medical Center Ulm, Department of Pediatrics, Ulm, Germany

¹³ Hôpital d'Enfants de la Timone Marseille, Marseille, France

¹⁴ Fondazione IRCCS Policlinico San Matteo, Pediatric Hematology-Oncology, Pavia, Italy

¹⁵ Jena University Hospital, Department of Pediatrics, Jena, Germany

¹⁶ IRCCS Ospedale Pediatrico Bambino Gesù, University La Sapienza, Department of Pediatric Hematology/Oncology, Rome, Italy

¹⁷ University Hospitals Leuven, Department of Pediatric Hematology-Oncology, Leuven, Belgium

¹⁸ Pediatric BMT Unit, Nantes, France

¹⁹ Turku University Hospital, Children's Hospital, Turku, Finland

²⁰ University of Heidelberg, Heidelberg, Germany

²¹ The Children's Hospital at Westmead, Division of Blood and Marrow Transplantation, Sydney, Australia

²² Ghent University Hospital, Department of Pediatric Hematology, Oncology and SCT, Ghent, Belgium

²³ University Children's Hospital Würzburg, Department of Pediatric Oncology, Würzburg, Germany

²⁴ The Queen Silvia's Hospital for Children and Adolescents, University of Göteborg, Pediatric Hematology and Oncology, Göteborg, Sweden

²⁵ Hospital Clínico Universitario Virgen de la Arrixaca, IMIB, University of Murcia, Barcelona, Spain

²⁶ Sheffield Children's Hospital, Department of Hematology and Oncology, Sheffield, United Kingdom

²⁷ Pediatric Hemato-Oncology Department, University Hospital, Rouen, France

²⁸ Bordeaux University Hospital, Pediatric BMT Unit, Bordeaux, France

²⁹ St. Anna Children's Hospital, Medical University Vienna, Vienna, Austria

³⁰ Imperial College London, Department of Medicine, London, United Kingdom

³¹ Medical University of Warsaw, Department of Hematology, Oncology and Internal Medicine, Warsaw, Poland

³² University of Regensburg, Department of Pediatric Hematology, Oncology & Stem Cell Transplantation, Regensburg, Germany

³³ Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain

³⁴ Goethe-Universität, Universitätsklinikum Frankfurt, Frankfurt, Germany

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* Correspondence and reprint requests: Maura Faraci, MD, Hematology/Oncology – Stem Cell Transplantation Unit, Istituto G. Gaslini, Largo G. Gaslini, 5; 16147 Genova, Italy.

E-mail address: maurafaraci@alice.it (M. Faraci).

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Gonadal impairment is an important late effect with a significant impact on quality of life of transplanted patients. The aim of this study was to compare gonadal function after busulfan (Bu) or treosulfan (Treo) conditioning regimens in pre- and postpubertal children. This retrospective, multicenter study included children transplanted in pediatric European Society for Blood and Marrow Transplantation (EBMT) centers between 1992 and 2012 who did not receive gonadotoxic chemoradiotherapy before the transplant. We evaluated 137 patients transplanted in 25 pediatric EBMT centers. Median age at transplant was 11.04 years (range, 5 to 18); 89 patients were boys and 48 girls. Eighty-nine patients were prepubertal at transplant and 48 postpubertal. One hundred eighteen children received Bu and 19 Treo. A higher proportion of girls treated with Treo in the prepubertal stage reached spontaneous puberty compared with those treated with Bu ($P = .02$). Spontaneous menarche was more frequent after Treo than after Bu ($P < .001$). Postpubertal boys and girls treated with Treo had significantly lower luteinizing hormone levels ($P = .03$ and $P = .04$, respectively) compared with the Bu group. Frequency of gonadal damage associated with Treo was significantly lower than that observed after Bu. These results need to be confirmed in a larger population.

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INTRODUCTION

Gonadal impairment represents an important late effect occurring after hematopoietic stem cell transplantation (HSCT), affecting the quality of life of young transplanted patients. The proportion and severity of gonadal late impairment depends on type and cumulative dosage of gonadotoxic chemotherapeutic agents, type of HSCT, age at HSCT, and additional use of total body irradiation (TBI) for conditioning or radiotherapy (cranial and pelvic radiotherapy) administered before HSCT [1]. Gonadal complications are recognized as a consequence of myeloablative conditioning regimens, in particular based on TBI or busulfan (Bu), and include hypergonadotropic hypogonadism and infertility. Radiation and/or alkylating agents induce irreversible ovarian failure in older girls because of the rapid decline in the number of follicles, whereas in younger girls a recovery of ovarian function may be observed [2–6]. In boys the spermatogenesis is more sensitive to radiation, and also low doses of radiotherapy may cause azoospermia [3].

The preparative regimen including Bu is associated with damage to the testicular function as reported by Sanders et al. [10] in 83% of patients. Although gonadal toxicity related to TBI has been frequently described [7–9], there are few reported studies on gonadal toxicity of Bu, in particular regarding prepubertal children. Sanders et al. [10] and Thibaud et al. [5] concluded that Bu induced a complete ovarian failure with extremely rare spontaneous recovery. Moreover, Sanders et al. [10] demonstrated that Bu administered at myeloablative doses (≥ 8 mg/kg) induced damage to testicular function and was associated with increased levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

Treoosulfan (Treo; L-treitol-1,4-bis-methanesulfonate) is a bifunctional alkylating agent with myeloablative and immunosuppressive effects. It is administered as a conditioning agent before HSCT in patients with malignant and nonmalignant diseases, such as primary immune deficiencies, metabolic disorders, hemoglobinopathies, histiocytic disorders, and autoimmune diseases [11–15]. Its limited extramedullary toxicity and the less frequent occurrence of graft-versus-host disease is reported in a retrospective European Society for Blood and Marrow Transplantation (EBMT) study including children who received Treo as preparative regimen [10].

To the best of our knowledge, no published studies have compared gonadal damage induced by Bu versus Treo in pre- and postpubertal children. This prompted us to perform a retrospective, multicenter study involving patients treated in

pediatric EBMT centers who received Bu or Treo as part of their preparative regimen for HSCT.

METHODS

This is a retrospective, multicenter, joint study conducted through the collaboration between the Pediatric Disease Working Party and the Transplant Complications Working Party of the EBMT society including pediatric patients treated with Bu or Treo at myeloablative doses (≥ 8 mg/kg and 36 to 42 g/m², respectively) between January 1992 and December 2012 and who did not receive chemotherapy and/or radiotherapy before HSCT. The underlying diseases included in the study that typically did not require gonadotoxic chemo- and/or radiotherapy before HSCT were primary immunodeficiency, Shwachman-Diamond syndrome, hemophagocytic lymphohistiocytosis, chronic myeloid leukemia, and juvenile myelomonocytic leukemia. Patient eligibility criteria also included age at HSCT between 5 and 20 years, follow-up ≥ 2 years, and being alive at the last follow-up. Patients who received chemo- and/or radiotherapy before HSCT, those affected by diseases causing iron overload such as hemoglobinopathies and primary diseases including gonadal failure, and patients who underwent pelvic surgery were excluded from the study.

Puberty was defined as the development of secondary sexual characteristics associated with skeletal and somatic growth; the time of onset of puberty was different in girls (normally from 8 to 13 years) and boys (from 9 to 14 years). In this study patients were stratified into 2 groups according to pubertal stage at HSCT, namely prepubertal and postpubertal patients. To simplify the analysis, we decided to include in the prepubertal group children aged between 5 and 14 years and in the postpubertal group those aged over 14 years. Spontaneous puberty (SP) was defined as the development of secondary sexual characteristic without hormonal replacement therapy (HRT).

The study was based on data from the EBMT database “Promise” and from those obtained through a questionnaire sent to all participating pediatric EBMT centers. The questionnaire contained questions about pubertal development and measurement of hormone levels at different time points of pubertal development, in particular for girls, the levels of LH, FSH, and 17 β -estradiol before HSCT and at the last follow-up and the inception of HRT administered for gonadal failure, and for boys, the levels of LH, FSH, and testosterone before HSCT and at the last follow-up. Hormone levels at the last follow-up were measured only in the postpubertal group. The evaluation of anti-Müllerian hormone and inhibin B as markers of gonadal function in girls and boys [16] was not recorded for the inhomogeneous determination of these markers in participating EBMT centers.

The primary endpoints of this study were to evaluate gonadal function after Bu versus Treo and to analyze the differences in the onset of SP and spontaneous menarche (SM) between patients treated with Bu versus Treo during prepubertal and postpubertal phases. Secondary endpoints were to analyze gonadotropin levels in these 2 groups of patients, number of patients on HRT, and results of sperm analysis.

All patients or legal guardians gave their informed consent to the use of their data in retrospective studies. The study was approved by the Institutional Review Board of the Pediatric Disease Working Party and by the Transplant Complication Working Party.

Statistical Analysis

Continuous data were summarized using median, interquartile range (IQR), minimum, and maximum values. All categorical/qualitative data were presented using absolute counts and percentage.

Patient-, disease-, and transplant-related characteristics of the 2 groups (Bu and Treo) were compared by using the chi-square or Fisher exact test for categorical variables and Wilcoxon test for continuous variables. Analysis was conducted in the 2 groups according to pubertal status at the time of HSCT. For measurement of hormone serum levels at last follow-up, only postpubertal patients were considered, and comparisons were stratified by gender. Two-sided $P < .05$ was considered statistically significant. Because of the limited number of patients, multivariate analysis was not conducted. Statistical analyses were performed with SPSS 24.0 (SPSS Inc., Chicago, IL) and R 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Of 137 patients included in this study, all were conditioned with a myeloablative dose of Bu or Treo administered in 25 pediatric EBMT centers between 1992 and 2012. The mean age at HSCT was 11.04 years (range, 5.02 to 18.55; IQR, 7.61 to 15.01). Of 137 patients, 89 (65%) were boys and 48 (35%) were girls. At the time of HSCT, 89 children (65%) were prepubertal (median age, 8.88 years; range, 5.02 to 14.3; IQR, 6.58 to 10.74) and 48 (35%) were postpubertal (median age, 15.83 years; range, 12.31 to 18.55; IQR, 14.97 to 17.11). The characteristics of these 137 patients are shown in Table 1. The difference in the number of patients who received Bu versus Treo from 1992 to 2007 and from 2008 to 2012 was statistically significant: patients 63 (53.39%) were treated with Bu versus 3 (15.79%) with Treo in the first period, and 55 (46.61%) were treated with Bu versus 16 (84.21%) with Treo in the second period ($P = .0023$).

Among the 89 prepubertal patients (62 boys and 27 girls), 77 (86.5%) received Bu (56 boys and 21 girls) and 12 Treo (13.5%; 6 boys). Median age at the last follow-up of the prepubertal patients was 17.03 years (range, 8.23 to 29.97; IQR, 14.49 to 19.66).

Among the 48 patients transplanted during the postpubertal period, 41 received Bu (85.5%; 21 boys and 20 girls) and 7 Treo (14.5%; 5 girls and 2 boys). Median age at the last follow-up in postpubertal patients was 23.9 years (range, 17.7 to 33.9; IQR, 21.9 to 25.9) in the Bu group and 23.9 years (range, 20.5 to 26.1; IQR, 21.6 to 24.7) in the Treo group ($P = .65$).

Data about SP were available for 85 of 89 prepubertal patients (58 boys and 27 girls) (Table 2). SP occurred in 64 of 85 prepubertal patients (75.3%; 50 boys [86.2%] and 14 girls [51.9%]). Considering the type of conditioning regimen, SP was achieved in 45 (80.3%) of 53 prepubertal boys who received Bu versus 5 treated with Treo (100%; $P = 1$) and in 8 (38.1%) of 21 prepubertal girls who received Bu versus 6 treated with Treo (100%; $P = .02$); the median age at SP in girls was 13 years (range, 11.5 to 16.9; IQR, 12 to 14.7).

Data about SM were available for 25 of 27 prepubertal girls (Table 3). SM occurred in 7 of 25 prepubertal girls (28%) and in 14 of 21 postpubertal girls (66.6%). Two of 20 prepubertal girls who received Bu had SM (10%) versus 5 of 5 patients treated with Treo (100%; $P < .001$). In the postpubertal group, 11 (64.7%) of 17 girls who received Bu had SM versus 3 (75%) of 4 who were treated with Treo ($P = .70$).

Table 1
Characteristics of the 137 Eligible Patients

	Population (N = 137)	Busulfan (n = 118)	Treosulfan (n = 19)	P
Puberty at HSCT				
No	89 (64.96)	77 (65.25)	12 (63.16)	.1957
Yes	48 (35.04)	41 (34.75)	7 (36.84)	
Median age at HSCT, yr (range) [IQR]	11.04 (5.02-18.55) [7.61-15.01]	10.7 (5-18.6) [7.6-15.1]	12.2 (6.1-18.4) [8.4-14.3]	.6161
Median age at diagnosis, yr (range) [IQR]	8.11 (0-18.04) [4.05-13.14]	7.7 (0-17.5) [4-13.1]	10.8 (1.3-18) [5.4-13.1]	.3486
Median interval between diagnosis and HSCT, mo (range) [IQR]	10.85 (3-207.15) [5.57-54.62]	11.8 (3-207.1) [5.5-56.8]	8.9 (3-129.3) [5.7-42.7]	.6074
<12 mo	71 (51.82)	59 (50)	12 (63.16)	.2867
≥12 mo	66 (48.18)	59 (50)	7 (36.84)	
Median years from HSCT to last follow-up (range) [IQR]	7.79 (2.07-22.69) [6.2-10.51]	7.8 (2.1-22.7) [6.3-11.2]	7 (3-9.1) [5.9-8.2]	.1286
Median age at last follow-up, yr (range) [IQR]	19.39 (8.23-33.86) [16.1-23.53]	19.2 (8.2-33.9) [16.2-23.6]	19.8 (12-26.1) [14.9-21.7]	.4057
Median year of HSCT (range) [IQR]				
1992-2007	66 (48.18)	63 (53.39)	3 (15.79)	.0023
2008-2012	71 (51.82)	55 (46.61)	16 (84.21)	
Gender				
Male	89 (64.96)	80 (67.8)	9 (47.37)	.0832
Female	48 (35.04)	38 (32.2)	10 (52.63)	
Diagnosis				
CML	41 (29.93)	38 (32.2)	3 (15.79)	
MDS	28 (20.44)	23 (19.49)	5 (26.32)	
Shwachman-Diamond syndrome	1 (.73)	1 (.85)	0 (0)	
CI	60 (43.7)	49 (41.52)	11 (57.89)	
HLH	7 (5.11)	7 (5.93)	0 (0)	
Source of stem cells				
BM	95 (69.34)	82 (69.49)	13 (68.42)	.5224
PB	34 (24.82)	30 (25.42)	4 (21.05)	
CB	8 (5.84)	6 (5.08)	2 (10.53)	
CR associated				
Cy (CED: 4000-6600 mg/m ²)	120 (87.5)	101 (85.5)	0	
Flu	32 (23.3)	17 (14)	15 (78.9)	
L-PAM	1 (7.2)	0	1 (5.2)	

Values are n (%) unless otherwise defined. CML indicates chronic myeloid leukemia; MDS, myelodysplastic syndrome; CI, congenital immunodeficiency; HLH, hemophagocytic lymphohistiocytosis; BM, bone marrow; PB, peripheral blood; CB, cord blood; Cy, cyclophosphamide; Flu, fludarabine; L-PAM, melphalan.

Table 2
Spontaneous puberty achievement in pre-pubertal children

No. of Patients (%)	Bu n (%)	Treo n (%)	P
85 prepubertal patients*	74	11	
64 Spontaneous puberty (75.3%)	53 (71.6%)	11 (100%)	.06
58 prepubertal boys	53	5	
50 Spontaneous puberty (86.2%)	45 (84.9%)	5 (100%)	1
27 pre pubertal girls	21	6	
14 Spontaneous puberty (51.8%)	8 (38.0%)	6 (100%)	.02

Bu= Busulfan; Treo=Treosulfan.

* In 4 of 89 prepubertal children, data were missing.

Table 3
SM in Prepubertal and Postpubertal Girls

No. of Girls	Bu *	Treo	P
25 prepubertal	20	5	
7 SM (28%)	2 (10)	5 (100)	.001
21 postpubertal	17	4	
14 SM (66.6%)	11 (64.7)	3 (75)	.70

Values are n or n (%).

* In 2 of 27 prepubertal girls, data were missing.

One hundred twelve patients were postpubertal at the last follow-up, and the results of hormone levels were available in 81 of these patients (72.3%) (Table 4). LH levels were significantly lower in both boys ($P = .03$) and girls ($P = .04$) who received Treo compared with those treated with Bu. Moreover, in girls, FSH levels were also statistically lower ($P = .02$) versus girls treated with Bu.

HRT was administered in 14 (51.8%) of 27 prepubertal girls and 13 (70%) of 21 postpubertal girls. Median age at HRT was 15.3 years (range, 12.8 to 18.5; IQR, 13.7 to 16.6). In 27 girls (62.7%), HRT was administered until the last follow-up.

Seventy-seven boys were postpubertal at the last follow-up, and data about sperm analysis were available for only 8

(17%). The results of sperm analysis were oligozoospermia in 5 patients, whereas in 3 the test result was missing.

DISCUSSION

This study is the first multicenter study comparing the effect of Bu and Treo on gonadal function. In the literature, although some studies have reported on gonadal damage secondary to Bu [17–21], data about gonadal damage in prepubertal children receiving Bu are lacking and studies on gonadal damage secondary to Treo are very few [22,23]. Studies on the gonadotoxic effects of the conditioning regimen on pubertal development and fertility often have the weakness of not adequately considering the additional gonadotoxic effect of chemotherapy and radiation administered before HSCT. Therefore, we chose to limit our study to patients affected by diseases that did not require chemo- and/or radiotherapy before HSCT.

Previous studies [2,3,24] reported a very high rate of gonadal dysfunction in patients treated with myeloablative conditioning regimen, more frequent hypergonadotropic hypogonadism in older patients, higher gonadotropin levels in females than in males, and major incidence of ovarian insufficiency with more advanced pubertal stage at HSCT. An important study by Sanders et al. [10] reported that only 1 of 73 females (1.3%) treated with Bu/cyclophosphamide had ovarian function recovery. More recently, Vatanen et al. [24] evaluated a group of 92 pubertal female survivors confirming that preserved ovarian function is more frequent in patients conditioned with cyclophosphamide alone in comparison with females prepared with either a TBI-containing (29%) or Bu-based (25%) regimen. Bresters et al. [2] demonstrated that almost half of the girls who were prepubertal or postpubertal at HSCT required hormonal induction of puberty and described the association of Bu with ovarian insufficiency in patients conditioned with chemotherapy alone. Panasiuk et al. [20] reported that girls treated with melphalan combined with fludarabine entered puberty spontaneously and required HRT to a lesser extent compared with girls receiving Bu/cyclophosphamide.

To the best of our knowledge, gonadal function in patients who received Treo has not been reported yet. An Italian study

Table 4
Post-Transplant Hormone Levels According to Conditioning Regimen

	Bu	Treo	P
All patients (n = 81)	66	15	
Age, yr	18.5 (11.4–32.6)	15.2 (12.6–24.4)	.04
LH, IU/L	6.6 (.1–73.2)	6.1 (.4–16)	.31
FSH, IU/L	9.8 (.2–141.1)	6.5 (2.5–32)	.08
Boys (n = 53)	47	6	
Age, yr	19 (11.4–30.5)	2.8 (.4–16)	.96
LH, IU/L	5.6 (.2–41.9)	5.5 (2.6–32)	.03
FSH, IU/L	8.1 (.6–108.7)	11.4 (.2–18)	.7
Testosterone, IU/L	13.5 (01–220.7)		.51
Girls (n = 28)	19	9	
Age, yr	31.7 (.1–73.2)	14.6 (12.6–20.4)	.10
LH, IU/L	43.9 (.2–141.1)	8.1 (1.9–15)	.04
LH before HRT, IU/L	31.7 (1.4–119) [20.1–46.1]	7.4 (.2–60) [4.1–9]	.018
FSH, IU/L	36.7 (10.7–301)	7.1 (2.5–15)	.02
FSH before HRT, IU/L	78.5 (4–200) [47.7–104.5]	6.6 (2.5–92) [4.7–8.3]	.0025
17β-Estradiol, IU/L	36.7 (10.7–301)	119.4 (18.4–279)	.35
17β-Estradiol before HRT, IU/L	12 (.2–94) [10–24.8]	60.3 (5–351) [10.8–119]	.16

Values are median (range); values in brackets are IQR.

on Treo-based conditioning regimen in children with sickle cell disease [15] reported an incidence of gonadal failure after Treo of 25%; however, the number of patients included in the study was too low to draw any conclusion. Recently, an experimental study [23] conducted in mice indicated that, unlike Bu, Treo has a differential gonadal toxicity profile, manifested as mild testicular toxicity contrasted with severe ovarian toxicity in both postpubertal and prepubertal mice, due to decrease of the number of primordial ovarian follicles.

In our study the number of patients treated with Bu was higher than those treated with Treo, because Treo is a relatively new treatment option adopted over the last 10 years. The discrepancy between patient sample size and the retrospective nature of the study represents a major limitation, and the low number of patients in the Treo group precludes any adjustment for potential confounding factors. Moreover, in this cohort of patients, most received Bu associated with cyclophosphamide, and the cyclophosphamide equivalent dose (CED) was between 4000 and 6600 mg/m², whereas those treated with Treo receiving fludarabine.

It is recognized that alkylating agents may interfere with fertility differently in males and females [25]. In particular, in males impaired spermatogenesis is described in patients who received CED > 4000 mg/m² [26], whereas in females the exposure to CED ≥ 8000 mg/m² was associated with premature ovarian failure [27]. In our study the CED was included between 4000 and 6600 mg/m². This observation could suggest that in our study CED did not influence the gonadal function in girls treated with Bu or Treo because the dose of CED was not high, whereas in boys CED could have interfered on the fertility also in our patients.

Regarding female patients, we observed that girls who received Treo during the prepubertal period more often reached SP compared with those treated with Bu ($P = .02$). This observation suggests that Treo has less impact on pubertal development. Also, SM occurred more frequently in girls treated with Treo compared with those treated with Bu ($P < .001$), which suggests that, contrary to experimental data, Treo seems to be less toxic to ovaries than Bu. Moreover, girls treated with Treo had statistically significant lower levels of LH, FSH, and 17β-estradiol compared with girls conditioned with Bu.

In boys, there was no statistically significant difference between the Treo and Bu groups in the development of SP, but the low number of patients did not allow a conclusion but only to report these results. Moreover, boys had statistically significant lower levels of LH in the Treo group versus the Bu group, whereas the difference in FSH and testosterone levels between the 2 groups was not significant.

In our study we compared gonadal function and its recovery after Bu versus Treo considering the periods between 1992 and 2007 and between 2008 and 2012. However, comparing the 2 conditioning regimens and selecting only patients transplanted between 2007 and 2012, the results were consistent with those of the entire population (data not shown). These data imply that Treo is less gonadotoxic than Bu in girls, whereas in boys its effect may be similar; however, further prospective studies are needed to confirm these results.

In conclusion, despite some limitations to our study, we can conclude that SP was more frequent in children treated with Treo compared with those conditioned with Bu. This result is particularly evident in girls in whom the occurrence of SM was more frequent after Treo, suggesting a greater ovarian reserve and a higher opportunity of being fertile. In boys, the levels of FSH were comparable in the 2 groups, suggesting similar effects of both agents on spermatogenesis. Further prospective

studies that include measurements of anti-Müllerian hormone and inhibin B and conducted in larger patient population are needed to better evaluate the impact of these 2 different regimens on fertility.

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