Biol Blood Marrow Transplant 22 (2016) 1627–1635

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Alternative Donors

Late Effects after Umbilical Cord Blood Transplantation in Very Young Children after Busulfan-Based, Myeloablative Conditioning

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Article history:
Received 27 February 2016
Accepted 27 May 2016

Key Words:
Late effects
Umbilical cord blood transplantation
Myeloablative conditioning
Busulfan

A B S T R A C T

Infants and young children who undergo allogeneic cord blood transplantation (CBT) are at increased risk for late effects because of exposure of developing organs to chemotherapy and radiation therapy typically used in transplant conditioning regimens. Busulfan (Bu)-based myeloablative regimens were developed to eliminate radiation exposure in these young children with the hope that late effects would be minimized. We now describe the late effects in 102 consecutive patients surviving a minimum of 5 years (median follow-up, 12.9 years) post-CBT. Patients were conditioned with high-dose chemotherapy using Bu-containing regimens. No patient received total body irradiation. The median age at transplant was 1 year (range, .1 to 2). Diagnoses included inherited metabolic diseases (59.8%), leukemia (17.6%), congenital immune deficiency (20.2%), bone marrow failure/myelodysplastic syndrome (3.9%), and hemoglobinopathy (2%). Among patients surviving 5 years, the overall survival rate at 10 years post-CBT was 93% (95% CI, 84.9 to 96.8). Virtually all patients (98%) experienced at least 1 significant late effect. Most (83.3%) experienced 2 or more late effects, and more than half of the patients (64.7%) experienced 3 or more late effects. The most commonly observed late effects included dental problems (92.2%), short stature (55.9%), cognitive deficits (53.6%), pulmonary dysfunction (18.6%), and abnormal pubertal development (27.9%). This is the first report of late effects of Bu-based conditioning in a cohort of very young patients at the time of transplant. These results will inform clinical care guidelines for long-term follow-up and add to the growing information regarding outcomes of hematopoietic stem cell transplantation.

INTRODUCTION

Over the past 3 decades, hematopoietic stem cell transplantation (HSCT) using umbilical cord blood (CBT) has been established as an effective therapy for pediatric patients with life-threatening malignant and nonmalignant conditions [1]. Many CBTs have been performed in infants and young children, who are at increased risk for late effects because of organ immaturity at the time of transplantation. Because of improvements in cord blood banking, preparative regimens, and supportive care, more of these patients are now surviving into adulthood. To date, the late outcomes of these patients and late effects occurring as a result of their transplantation therapy have not been reported in a systematic fashion.

Late effects occur due to multiple factors, including age at transplant, primary diagnosis, comorbidities, pretransplant therapy, conditioning regimens, development of graft-versus-host disease (GVHD), and other transplant-related complications [2] Most reports of late effects to date have focused on outcomes in older children and adults [2-17]. Most information published to date regarding late effects after HSCT in children has focused on total body irradiation (TBI)-containing conditioning regimens, where the most commonly observed late effects include growth hormone deficiency and short stature, cognitive dysfunction, abnormal puberty, and thyroid dysfunction [2-7,9,11-15,18,19]. Over the past 2 decades, alternative busulfan (Bu)-based,

Financial disclosure: See Acknowledgments on page 1634.

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http://dx.doi.org/10.1016/j.bbmt.2016.05.024
1083-8791/

myeloablative chemotherapy regimens have been used to eliminate radiation exposure and its consequences, particularly in very young patients.

There remains a paucity of data regarding late effects in very young children undergoing HSCT, particularly in those prepared for transplantation with myeloablative, non-TBI preparative regimens. Elucidating these effects will allow for the development of a strategic, evidence-based algorithm for surveillance and long-term care for these patients with the goal of preserving organ function to the fullest extent possible. We now report the results of a single-center, retrospective review of very young patients who underwent CBT using Bu-based conditioning regimens, describing the characteristics of the cohort, overall survival, and incidence of key late effects.

METHODS

Study Design

The study was designed as a single-center, retrospective study reviewing clinical data routinely collected by the Pediatric Blood and Marrow Transplant Program at Duke University Medical Center. Patients were selected from a cohort of consecutively transplanted patients, who each received a single umbilical cord blood unit at less than 2 years of age from September 1993 to August 2008 after chemotherapy-based cytoreduction.

Eligibility

Eligibility criteria included age 2 years or less at transplant, stem cell source related or unrelated umbilical cord blood, myeloablative Bu-based preparative regimen, survival to a minimum of 5 years post-transplant, and transplant date before August 2008. Criteria for exclusion included TBI-containing or reduced-intensity conditioning and non–cord blood graft sources. Three patients who had received craniospinal irradiation or later received TBI were excluded. All patients were enrolled in an Institutional Review Board–approved protocol or treatment plan for transplant, and written informed consent was obtained from the parents or legal guardians of all patients before the initiation of conditioning therapy. Institutional Review Board approval was also obtained for this retrospective review. Information regarding the transplantation, supportive care, and early transplant courses for a subset of these patients was previously reported in articles describing outcomes of the Cord Blood Transplantation Study (n = 24) and in other reports from Duke University Medical Center [20-25].

Data Collection

Patients were routinely evaluated for follow-up transplant care at Duke at a minimum of every 3 to 6 months for the first year, every 6 months for the second year, and then on an annual basis. Patients with active issues were seen more frequently. Medical records were reviewed to collate data from these follow-up visits, including physical examinations, and assessments of disease status, growth, and organ function were performed (Table 1).

Cord blood unit data were provided by the cord blood bank supplying the unit for transplantation and post-thaw characteristics and dosing by the Duke Stem Cell Transplant Laboratory. Confirmation of donor cell chimerism was performed using fluorescence in situ hybridization or restriction fragment length polymorphism. Grading of GVHD was performed according to conventional criteria [26,27]. Duration of steroid exposure was defined as the period during which patients received steroid doses of 1 mg/kg daily or higher. Pulmonary toxicity and neurocognitive deficits were graded according to the National Cancer Institute/National Institutes of Health Common Terminology Criteria for Adverse Events [28].

Precocious puberty was defined as Tanner stage $\geq$ 2 development by 7 years for females and 9.5 years for males. Delayed puberty was defined as Tanner stage 2 or less development by age 13 years for females and 14 years for males. Long-term sequelae of primary diseases but not transplantation were not included in scoring of late effects. Causes of death were coded according to the algorithm published in 2007 by Copelan, et al. [29].

Statistical Analysis

Descriptive statistics are presented for baseline characteristics and late effects including absolute and relative frequencies for categorical data and the 5-number summary for continuous data. A Kaplan-Meier survival estimate is presented at 10 years post-transplant. Cumulative incidence for observed late effects with death as a competing risk is presented. Further analyses explored associations between possible predictors and late effects. Predictors included age at transplant, gender, diagnosis (malignant or nonmalignant), Lansky performance score, duration of steroid therapy, duration of immunosuppressive therapy, and chronic GVHD (cGVHD) 2 years post-transplant (for late effects other than cGVHD at 5 years). Duration of steroid therapy was examined as both continuous and categorical, with cut-offs at 6 months, 1 year, and 2 years. Univariate Cox regression models explored individual associations between each predictor and late effect, where an event date was known. If there was evidence of significant univariate associations, a multivariate model was constructed to further examine these associations in the presence of other potential confounders. If the event date was unknown for a particular late effect, a logistic regression model was used to explore associations at the univariate and multivariate levels.

Multivariate models were then reduced using backward selection with selection criterion of $P < .05$. In general, $P < .05$ was considered to be statistically significant. For patients with nonmetabolic diagnoses, height data were evaluated by using growth charts provided by the World Health Organization (up to 2 years of age) and the Centers for Disease Control and Prevention (2 to 20 years of age). Median height standard deviation (SD) scores were calculated using the LMS method based on the use of Box-Cox transformations (http://www.cdc.gov/growthcharts/percentile_data_files.htm). Height SD scores were not calculated for patients with metabolic disorders because no published reference height ranges exist for inherited metabolic disease at this time. All analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC).

Table 1
Long-Term Follow-up Visit Evaluations

Studies Performed
Physical examination
Assessment of growth curve
Tanner staging
Complete blood count
Complete metabolic panel
Chimerism
Cellular and humoral immune reconstitution panels
Immunoglobulins
Thyroid hormone levels: thyroid-stimulating hormone, thyroxine (T_4), triiodothyronine (T_3)
Reproductive hormone levels: estradiol/testosterone, follicular-stimulating hormone, luteinizing hormone
Echocardiogram
Electrocardiogram
Spirometry
Diffusion capacity of carbon monoxide
Ophthalmologic examination
Audiologic evaluation
Dental examination
Neurocognitive testing
Additional disease-specific evaluations for malignancies: disease surveillance (ie, PCR)
Additional disease-specific evaluations for metabolic diseases: magnetic resonance imaging, electroencephalogram, electromyelogram, nerve conduction studies, brainstem auditory-evoked response, visual-evoked potentials

RESULTS

Study Population

In this study we report the late effects observed in 102 patients under 2 years of age treated at Duke University Medical Center from September 1993 to August 2008 who survived more than 5 years after CBT after cytoreduction with Bu-based myeloablative, non-TBI containing, preparative regimens. Patient, donor, and transplant characteristics of the study cohort are shown in Table 2. The median ages at transplant and follow-up were 1 year (range, .1 to 2) and 13.8 years (range, 7.7 to 23.8), respectively. Most patients underwent CBT for nonmalignant diseases (82.4%), and more than half of patients with nonmalignant diagnoses were transplanted for inherited metabolic diseases (59.8%). The most common inherited metabolic diseases were Krabbe disease (globoid cell leukodystrophy, n = 18) and mucopolysaccharidosis (n = 35), of which most cases were type 1

Table 2
Patient, Donor, and Transplant Characteristics (N = 102)

Characteristics	Value
Median age at transplant, yr (range)	1 (.1-2)
Median age at review, yr (range)	13.8 (7.7-23.8)
Median time from transplant at analysis, yr (range)	12.9 (7.2-22)
Diagnoses	
Malignant	18 (17.6)
ALL	8 (7.8)
AML	5 (4.9)
Other	5 (4.9)
Nonmalignant	84 (82.4)
Immunodeficiency	17 (20.2)
Metabolic	61 (59.8)
Bone marrow failure	4 (3.9)
Hemoglobinopathy	2 (2)
Gender	
Male	62 (60.8)
Race	
White	84 (82.4)
African American	6 (5.9)
Other	12 (11.8)
CMV status negative	80 (78.4)
Pretransplant performance score: <80	11 (10.8)
Donor Characteristics	
HLA matching	
6/6	12 (11.8)
5/6	46 (45.1)
4/6	44 (43.1)
Median cell dose (range)	
Pre-cryopreservation, $\times 10^7$/kg	12.6 (1.2-50.3)
Infused, $\times 10^7$/kg	9.9 (1.5-32.4)
Infused CD34, $\times 10^5$/kg	3.3 (.8-104.8)
Transplant characteristics	
Conditioning regimen	
Bu/Cy/ATG	75 (73.5)
Bu/Mel/ATG	15 (14.7)
Bu/Flu/Cy/ATG	5 (4.9)
Bu/Flu/Mel/ATG	3 (2.9)
Bu/Cy/VP-16/ATG	3 (2.9)
Bu/Mel	1 (1)

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CMV, cytomegalovirus; ATG, antithymocyte globulin; Mel, melphalan; Flu, fludarabine; VP-16, etoposide.
Values are number of cases with percents in parentheses, unless otherwise indicated.

(Hurler syndrome, n = 29). Most patients were cytomegalovirus negative pretransplant (78.4%).

Transplant Preparation and Graft Characterization

Conditioning regimens did not include TBI and were based on patient age, diagnosis, and disease state. All patients received Bu-based regimens, with most receiving either Bu/cyclophosphamide (Cy)/antithymocyte globulin (73.5%) or Bu/melphalan/antithymocyte globulin (14.7%). The remaining patients were treated with combinations of Bu with fludarabine, melphalan, antithymocyte globulin, etoposide, and/or Cy. Bu was administered enterally in 79 patients (77.5%) and intravenously in 23 patients (22.5%) at a median dose of 31 mg/m^2 (range, 15.6 to 62.5) every 6 hours on days −9 to −6, with goal steady-state values of 600 to 900 ng/mL. GVHD prophylaxis consisted of cyclosporine/steroids (73.5%), cyclosporine/mycophenolate (23.5%), cyclosporine/steroids/methotrexate (n = 1), or cyclosporine alone (n = 2).

All patients, given their young age and small size, received high cell doses from the cord blood graft. The median total nucleated cell dose from the pre-cryopreservation units was 12.6 × 10^7 cells/kg (range, 1.2 to 50.3). A total of 9.9 × 10^7 nucleated cells/kg (range, 1.5 to 32.4) and 3.3 × 10^5 CD34 cells/kg (range, .8 to 104.8) were infused. Most patients received cord blood units mismatched at 1 (45.1%) or 2 (43.1%) HLA loci from unrelated (n = 100) or related (n = 2) cord blood donors (Table 1).

Transplant Outcomes

The overall incidences of acute GVHD grades II or higher and cGVHD beyond skin involvement were 11.8% and 9.8%. After 5 years, 18 patients (17.6%) continued to have active cGVHD (17 limited, 1 extensive). Four (3.9%) required ongoing treatment with systemic steroids, 6 (5.9%) required other systemic anti-GVHD therapy, and 8 patients (7.8%) had limited GVHD of the skin requiring only topical agents. Four patients (3.9%) experienced graft failure, 2 of whom successfully engrafted after undergoing subsequent HSCT. All other patients were engrafted with full donor chimerism.

Survival

Overall survival of the study cohort (the 102 patients surviving ≥5 years post-UCBT) was 93% (95% confidence interval [CI], 84 to 9-96.8) at 10 years post-transplant. There were 8 late deaths, none of which was transplant-related. All late deaths occurred in patients with metabolic diseases, 6 of whom died of progression of their underlying disease and 2 who died of unusual events. One patient with Hurler syndrome suffered a complication of mitral valve replacement surgery and 1 patient with Krabbe disease developed a reaction to ketamine after surgical tendon release. For surviving patients, median performance status at last follow-up (Lanksy/Karnofsky score) was 80% (range, 10% to 100%) for patients with metabolic diagnoses and 100% (range, 70% to 100%) for patients with nonmetabolic diagnoses.

Dental Outcomes

Abnormal dentition was the most common late effect observed (92.2%, Table 3). There was documentation of absent adult teeth in 30 patients (29.4%). Other commonly occurring transplant-related dental issues included microdontia, enamel hypoplasia, and extensive caries/decay, for which many survivors have required extensive restoration as older children/adolescents. One such example of these dental problems is illustrated in Figure 1.

Endocrine Outcomes

Short stature

Defined as height more than 2 SDs below age- and gender-matched mean, short stature was documented in 55.9% of patients (Table 3). Eighty-one percent of patients with short stature had metabolic disorders. In univariate models, diagnosis type (metabolic versus nonmetabolic, $P < .0001$), duration of steroid therapy ($P < .01$), duration of immunosuppression ($P = .01$), and presence of cGVHD beyond 2 years post-transplant ($P < .01$) were significant predictors of short stature. Multivariate analysis revealed an association between diagnosis and short stature in a full model. After backward selection, diagnosis ($P < .0001$) and duration of immunosuppression remained in the model. Each year of immunosuppressive therapy was associated with a 44% increase in short stature (95% CI, 1 to 2.1; $P = .045$). Fourteen patients (13.7% of the cohort) received growth hormone replacement (Table 3); 11 of these patients had inherited metabolic diseases.

Median height SD scores for all patients with nonmetabolic diagnoses were −.6 (range, −3.2 to +1.3) and −.5

Table 3
Observed Late Effects

Late Effects	Metabolic (n = 61)	Nonmetabolic (n = 41)	Total (n = 102)
Dental problems	60 (98.4%)	34 (82.9%)	94 (92.2%)
Short stature	46 (75.4%)	11 (26.8%)	57 (55.9%)
GH therapy	11	3	14
Abnormal pubertal development	8 (32%*)	4 (22.2%*)	12 (27.9%*)
Assessable	25	18	43
Delayed puberty	2	4	6
Precocious puberty	6	—	6
Normal development	17	14	31
Prepubertal and normal	30	16	46
Unknown	6	7	13
Thyroid dysfunction	9 (14.8%)	9 (22%)	18 (17.6%)
Cognitive dysfunction	43 (70.5%)	9 (25%)	52 (53.6%)
Moderate	27	9	36
Severe	8	—	8
Profound	8	—	8
Unknown	—	5	5
Pulmonary dysfunction	15 (25%)	4 (9.8%)	19 (18.6%)
Grade 2 dysfunction	10	4	14
Grade 3 dysfunction	5	—	5
Seizures	9 (14.8%)	3 (7.3%)	12 (11.8%)
Cataracts	2 (3.3%)	5 (12.2%)	7 (6.9%)
Autoimmune cytopenias	2 (3.3%)	—	2 (2%)

GH indicates growth hormone.
Grade 2 dysfunction, FVC or FEV_1 74% to 50% predicted; grade 3 dysfunction, FVC or FEV_1 < 50% predicted.
* Percent of assessable patients.

(range, −2.4 to +.8) at 5 and 10 years post-transplant. Median height SD scores for those patients with nonmetabolic diagnoses who received growth hormone therapy were −.51 (range, −1.25 to −.05) at 5 years and −.84 (range, −1.08 to −.13) at 10 years post-transplant. For patients with nonmetabolic diagnoses who did not receive growth hormone therapy, median height SD scores were −.65 (range, −3.24 to +1.26) and −.53 (range, −2.41 to +.79) at 5 and 10 years post-transplant (Figure 2). The incidence of decreased bone mineral density could not be assessed in this cohort because screening was not performed frequently or without clinical suspicion. Dual-energy x-ray absorptiometry was performed in only 5 patients, all of whom had decreased bone mineral density.

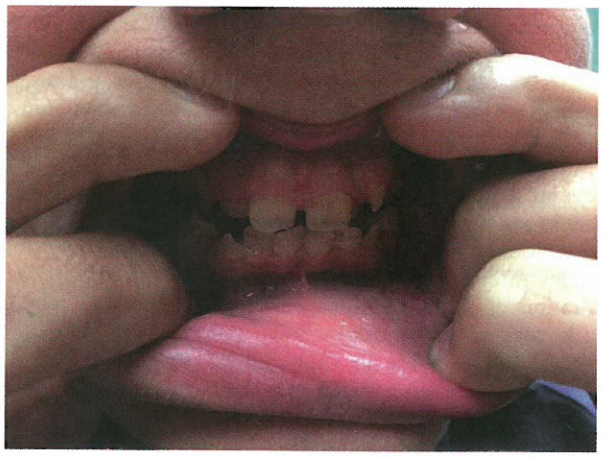

Figure 1. Microdontia and enamel hypoplasia in a 13-year-old patient who was 1 year of age at transplant.

Abnormal pubertal development

Forty-six patients (45.1%) remained prepubertal by virtue of age, and 43 (42%) were assessable for pubertal development by virtue of age. Among these 43, 31 (72%) had normal pubertal development. Precocious puberty was seen in 6 patients (5.9%), and 6 (5.9%) had delayed development (Table 3). Of those with abnormal puberty (n = 12), 75% were female and 25% were male, and all 6 patients with pubertal delay were female. Fifty-nine percent of females and 85.7% of males assessable for puberty achieved normal spontaneous pubertal development. Patients with precocious puberty were treated with the gonadotropin-releasing hormone agonist, leuprolide acetate. Those with delayed puberty were all female, and all but 1 were treated with estrogen therapy.

Thyroid disease

The cumulative incidence of thyroid disease at 5 and 10 years post-CBT was 11.8 (95% CI, 5.6 to 18) and 17.5 (95% CI, 9.9 to 25.1; Table 2, Figure 3A), respectively. Most of those affected had overt hypothyroidism (88.9%). None of the patient or treatment characteristics evaluated was significantly predictive of thyroid disease in either univariate or multivariate analyses. No benign thyroid nodules or thyroid malignancies have been observed in this cohort to date. Patients with hypothyroidism were treated with levothyroxine replacement, and 1 patient with hyperthyroidism received methimazole.

Neurologic Outcomes

Fifty-four percent of patients in this cohort had cognitive deficits of moderate or worse severity at last follow-up. Moderate deficits, defined as interfering with work/school/life performance and requiring part-time specialized resources, were seen in 37.1% of the cohort. Moderate deficits were observed in 44.3% of those with metabolic disorders and in 25% of those with nonmetabolic disorders. Severe deficits, defined as significantly impairing work/school/life performance, were seen in 8.2% of the cohort. Profound deficits, defined as requiring full-time specialized resources or institutionalization, were observed in 8.2% of the cohort. Severe and profound deficits were only seen in those who underwent transplant for metabolic disorders (Table 2). Forty-three (70%) of the metabolic patients had issues with cognitive dysfunction that were moderate in 27 and severe or profound in 16. This is in contrast to 9 nonmetabolic patients (25%) who had moderate cognitive dysfunction.

In univariate analyses, gender, diagnosis, and duration of steroid therapy (when evaluated categorically, not continuously) were significant predictors of cognitive disturbance. Males were 3.1 times as likely to demonstrate cognitive deficits compared with females (95% CI, 1.3 to 7.2; $P < .01$), and patients with metabolic diseases were 7.2 times as likely to do so compared with patients with nonmetabolic diagnoses (95% CI, 2.8 to 18.2; $P < .0001$). Patients who received steroid therapy for 6 months or greater had 5.5 times the odds of developing cognitive disturbance than those who received steroids for less than 6 months (95% CI, 1.6 to 18.4; $P < .01$). Multivariate analysis demonstrated significant associations between gender and diagnosis with cognitive deficits in full and reduced models after adjusting for other potential confounders. Odds of cognitive disturbance were 4.1 times higher in male patients

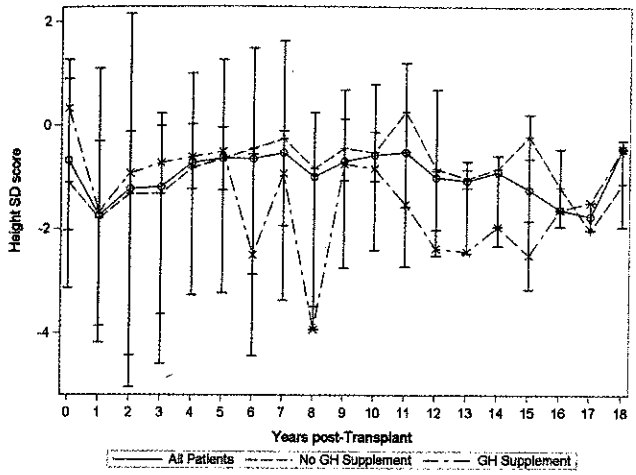

Figure 2. Median height SD scores and their corresponding ranges. GH indicates growth hormone.

(95% CI, 1.4 to 11.9; $P = .01$) and 8.3 times higher in patients with metabolic diseases (95% CI, 2.6 to 26.4; $P < .01$). Twelve percent of the cohort had seizures after 5 years post-transplant; of these, 1 patient had seizures before transplant and 9 (75%) had metabolic disorders.

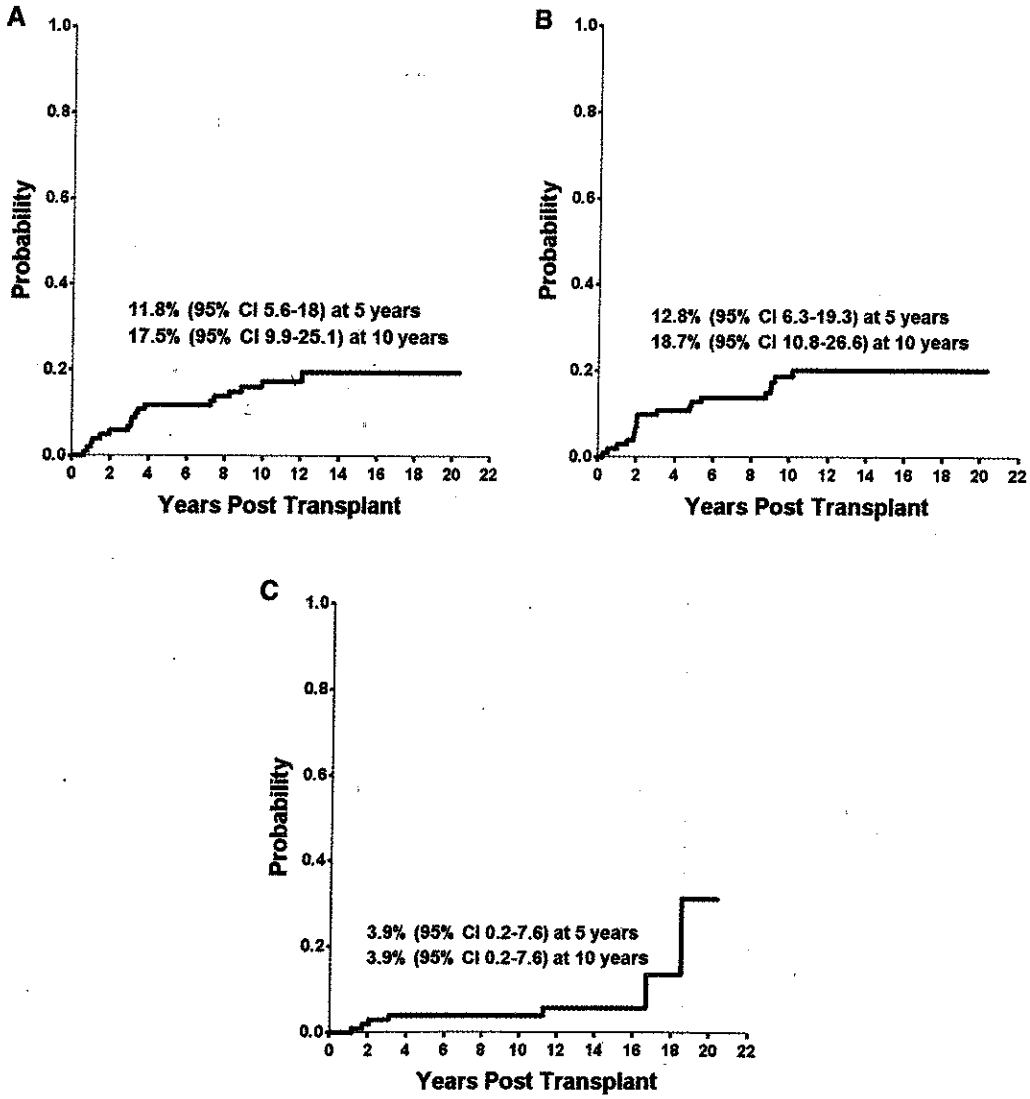

Figure 3. Cumulative incidences of late effects. (A) Thyroid dysfunction. (B) Pulmonary dysfunction. (C) Cataracts.

Pulmonary Outcomes

The cumulative incidence of grades 2 or higher pulmonary disease, defined as forced expiratory volume in 1 second (FEV_1) or forced vital capacity (FVC) less than 75% of predicted, was 18.7% at 10 years (95% CI, 10.8 to 26.6; Figure 3B). Of those, 73.7% had grade 2 disease (FEV_1 or FVC < 75% to 50% predicted) and 26.3% had grade 3 disease (FEV_1 or FVC < 50% to 25% predicted) at maximum severity (Table 3). Six patients had both obstructive and restrictive disease. Age at transplant, gender, diagnosis (metabolic versus nonmetabolic), pretransplant performance score, presence of cGVHD beyond 2 years post-transplant, duration of steroid therapy, or duration of immunosuppression were not found to be significant predictors for pulmonary disease. Thirteen patients carry a diagnosis of asthma (13.1%). Diffusion capacity for carbon monoxide was reported in only 22 patients, none of which was < 60%. No cases of bronchiolitis obliterans were seen in this cohort.

Vision

Seven patients developed cataracts, resulting in a cumulative incidence of 3.9% (95% CI, .2 to 7.6) at 10 years (Table 2, Figure 3C). Two of these patients had received steroids before umbilical CBT for leukemia and 1 for hemophagocytic lymphohistiocytosis. The duration of steroid exposure after umbilical CBT for patients who developed cataracts ranged from .3 to 7.2 years.

Immune/Autoimmune Outcomes

Two patients (2%) experienced immune-mediated cytopenias, unrelated to GVHD, after 5 years post-transplant (Table 3). One patient developed both rheumatoid arthritis and recurrent nodular vasculitis. Intravenous immunoglobulin replacement for hypogammaglobulinemia was continued beyond 5 years post-transplant in 9.9%. No serious or chronic infections occurred after 5 years post-transplant.

Other Late Effects

Only 1 patient developed disease-unrelated cardiac dysfunction; this patient with Krabbe disease underwent surgical correction of a subaortic stenosis with ventricular hypertrophy. Chronic kidney disease was seen in only 1 patient; this patient with Hurler syndrome developed renal insufficiency in association with treatment for autoimmune cytopenias. One patient who underwent umbilical CBT for leukemia later developed Henoch-Schonlein purpura.

DISCUSSION

Incidence and Characterization of Late Effects

The main focus of this study was to document the incidence of late effects after CBT at a very young age and any associated risk factors identified. Late effects associated with growth and development, organ function, and disease-related comorbidities were evaluated. In our series, 98% of patients experienced at least 1 late effect; 2 or more late effects were documented in 83.3% of patients and 3 or more in 64.7%. The most commonly observed late effects among the patients with metabolic diseases included dental problems (98.4%), short stature (75.4%), cognitive deficits (70.5%), and pulmonary dysfunction (25%). Among patients with nonmetabolic diseases, those most commonly observed were dental problems (82.9%), short stature (26.8%), and cognitive problems (25%).

HSCT is a curative option for infants and very young children with high-risk leukemia and certain genetic diseases. A high incidence of late effects has been previously reported in series of patients transplanted at a young age and prepared for transplantation with TBI-containing regimens [2-4,6-9,11-16,18,30]. We hypothesized that avoidance of TBI in the preparative regimen would lessen the late effects observed in this population. Our transplant center has used high-dose chemotherapy to prepare very young children for transplantation for over 20 years. In this report we examined the incidence of late effects in children surviving more than 5 years post-transplantation and showed that most patients exhibited more than 2 late effects by 5 to 10 years post-transplant.

Effects on dentition were nearly universal in this cohort, an effect that is uniquely associated with the use of chemotherapy in the very young child [7,18]. Both primary and permanent teeth that have not yet erupted are susceptible to damage by preparative transplant regimens, and many patients with transplant-related dental issues go on to require dental restoration procedures. Dental examination should be performed routinely by a dentist who is comfortable with pediatric patients and complex medical issues. It is important to advise families and dentists caring for survivors to perform regular dental care, seal permanent teeth as they erupt, and not remove primary teeth without confirming that the secondary tooth is present under the gum and developing normally.

Infants and young children, naturally undergoing growth and development of all major organs, are particularly susceptible to late effects after HSCT. Problems with growth and puberty have been well documented in children who undergo transplant after TBI [3,5,7,12,13]. Short stature and growth hormone deficiency have been reported in 20% to 85% of patients, varying by age at transplant, preparative regimen, and definitions of growth impairment [5,12]. Not surprisingly, abnormalities of skeletal growth were seen in a large proportion of children in this series, and duration of immunosuppressive therapy was found to be predictive of short stature. However, patients with nonmetabolic diagnoses had height SD scores lower than the median score of −1.5 previously reported in infants who received TBI before transplantation for acute lymphoblastic leukemia who received TBI [19]. Similar growth analysis was unable to be performed for patients with metabolic disorders because no published reference ranges exist, but metabolic diagnoses were associated with increased risk of short stature. This is not surprising because short stature is a manifestation of many inherited metabolic diseases. Long-term follow-up evaluations should include close monitoring of growth parameters and referral to an endocrinologist if short stature is identified. Growth hormone replacement has been associated with significantly improved final height in some children, and puberty may be medically postponed to allow for further growth before epiphyseal closure [13].

Delayed pubertal development has been described after both TBI-based regimens and Bu/Cytoxan [12]. In our cohort, 59.1% of females assessable for puberty achieved normal spontaneous pubertal development. This is slightly higher than the rates of normal spontaneous puberty among females in both the Bu/Cy (52%) and fractionated TBI groups (51%) reported by Sanders [12]. We observed a higher rate of normal spontaneous puberty in assessable males at 85.7% compared with the 72% in the Bu/Cy group and 55% in the fractionated TBI group reported by Sanders. This discrepancy is likely attributable at least in part to the younger age at transplantation of patients in this series (all <2 years of age

versus <12 years of age). Most of our patients (72%) had normal pubertal development.

Several prior studies have reported thyroid dysfunction and thyroid malignancies after HSCT but with variable incidences and follow-up time [5]. Sanders et al. [14] reported an incidence of abnormal thyroid function in 23% of their cohort of 108 patients who received non-TBI conditioning with Bu/Cy or Bu/melphalan and 56% of 61 patients who received Bu/TBI; there were 18 cases of thyroid tumors, all of which followed TBI-based regimens. We report a similar incidence of abnormal thyroid function at 17.6% and no thyroid tumors in this cohort, although follow-up time was shorter in this study.

The incidence of cognitive deficits in this cohort, particularly the severe and profound deficits observed in patients with metabolic diseases, highlights the need for regular performance of neurodevelopmental evaluation. Gender, nonmalignant diagnosis, and duration of steroid therapy were predictive of cognitive disturbance. Neurocognitive dysfunction and mood disorders have been described after transplant using TBI-containing regimens, but methodologic challenges have limited the knowledge base thus far [9]. Phipps et al. [10] have reported that TBI exposure and presence of GVHD are associated with risk of cognitive decline, although the effect size was small; younger age at transplant appeared to be predictive of neurocognitive deficits in 1 report but not in a subsequent report from the same cohort. In a recent report by Willard et al. [17], of 183 pediatric patients who underwent cognitive assessments after HSCT, the youngest patients (<3 years) who received TBI demonstrated significantly lower intelligence quotients than did those who did not receive TBI. Identification of those at risk and early diagnosis of neurocognitive deficits allow for potentially beneficial interventions such as adaptive therapies, education, and resources.

Although direct comparisons with prior reports are not possible in each instance because of varying ages at transplant and conditioning regimens, we compared our observed late effects with previously published outcomes in children prepared for HSCT with TBI. Short stature was seen less frequently than previously reported after TBI (54% to 84%) and to a lesser degree of severity as described above [7,19]. Gonadal failure was also less common in males in this cohort than in males who had received TBI; for females, the frequency of gonadal failure was similar to that after TBI as reported by Sanders et al. [12]. Thyroid dysfunction was less commonly observed than has been previously reported after Bu/TBI [14]. Pulmonary dysfunction was less common at 18.6% in this cohort than had been previously reported by Hoffmeister et al. [31] in survivors of pediatric HSCT in general (57%) and in those who received single-fraction TBI (72%) and fractionated TBI (28%). Frequency of pulmonary dysfunction was similar to that reported in those prepared with non–TBI-containing regimens, although many of these patients had not received Bu (17% with restrictive lung disease and 21% with obstructive lung disease) [4,8,31].

Other late effects described after HSCT in children and adults receiving various preparative regimens that have commonly included TBI include chronic kidney disease and cardiovascular disease. Chronic kidney disease, reported in 4% to 44% of children and adults after HSCT, was not prevalent in this cohort [8,32]. Cardiovascular disease has been reported predominantly after HSCT in adults and in children transplanted for malignancies with associated anthracycline exposure; cardiovascular disease was also not prevalent in this cohort [8]. There were no cases of chronic liver dysfunction. None of the patients in this cohort required solid organ transplant related to late effects of myeloablative chemotherapy for umbilical CBT. No second malignancies have been documented in the cohort at time of review.

One limitation of this report is that most of the patients reported underwent umbilical CBT for nonmalignant disease, particularly metabolic disease, which could potentially bias our ability to assess neurologic, cardiac, and orthopedic outcomes. There is not an evidence-based way to document the effects of their primary disease on surviving patients. However, to the extent we could, we have attempted to separate these disease manifestations from late effects after umbilical CBT. In this report, we did not score known sequelae of disease in untreated patients; for example, valvular cardiac disease in mucopolysaccharidosis was not scored as a late cardiac effect. Furthermore, the overall incidence of certain late effects in our cohort is not yet known because these patients were only followed for a median of almost 13 years at the time of this report. For instance, we may have underestimated the incidences of cataracts and second malignancies. Similarly, the incidence of infertility could not be estimated because very few patients in this cohort have reached childbearing age. We will continue to collect data as we follow these patients long term.

The results of this study, where dental, endocrine, and cognitive effects were common, illustrate that avoiding TBI in the very young undergoing HSCT may decrease but does not eliminate late effects. Long-term survivors of Bu-based regimens in infancy and early childhood require regular follow-up to evaluate for and treat the consequences of late effects. This is the first report of late effects of Bu-based conditioning in a large cohort of patients who were very young at time of CBT. These results will inform clinical care and guidelines for long-term follow-up and add to the growing information regarding late effects of HSCT in general.

Recommendations for Screening for Late Effects

Early recognition and treatment of late effects may help optimize outcomes for children who receive a Bu-based conditioning regimen at less than 2 years of age. In Table 1 we present our current model for annual evaluations and propose that this be used to guide clinicians providing long-term post-transplantation follow-up for those who have undergone myeloablative chemotherapy-based regimens at a very young age (additional screening for disease-related sequelae may be necessary, examples of which are included in Table 1). We recommend the following regarding the evaluation and management of specific late effects observed in this study.

Dental health

Dental examination should be performed every 6 months. Permanent teeth should be sealed as they erupt, and primary teeth should not be removed without confirming that secondary teeth are present under the gum and developing normally.

Growth

Height and weight curves must be followed closely after umbilical CBT. Bone age films should be used to evaluate skeletal maturity and thus potential for further growth. Growth hormone replacement therapy should be considered for those patients with short stature who have not yet

undergone epiphyseal closure. These patients should be referred to a pediatric endocrinologist. Reproductive hormone suppression can be used to medically postpone puberty until patients have reached goal height.

Neurocognitive function

Patients should undergo developmental evaluation and neurocognitive evaluation annually, and school performance should be followed closely. Information obtained should be used to guide additional therapies (ie, physical, occupational, and speech therapies), resources, and learning/testing accommodations.

Pulmonary function

Annual evaluation should include spirometry and diffusion capacity of carbon monoxide, when age and condition permits. For very young, uncooperative, or neurologically impaired patients, measurement of respiratory rate and pulse oximetry will suffice. Patients with obstructive or restrictive lung disease should be referred to a pulmonologist for recommendations regarding management.

Pubertal development

Tanner staging should be evaluated and documented annually. Patients with abnormal pubertal development should be referred to a pediatric endocrinologist. For patients with precocious pubertal development, reproductive hormone suppression can be used to delay puberty until they have had adequate skeletal growth and are deemed ready from a psychosocial standpoint. Those with delayed pubertal development may benefit from estrogen/androgen replacement therapy.

Thyroid function

Thyroid hormone levels should be obtained annually. Patients with abnormal thyroid function should be referred to an endocrinologist. Those with hypothyroidism should be treated accordingly with thyroid hormone replacement. Hyperthyroidism may be treated medically, with radioablation, or surgically depending on other patient factors. Palpation of the thyroid is an important part of the follow-up physical examination. Enlargement, nodularity, or lesions of the thyroid gland warrant prompt evaluation for malignancy.

ACKNOWLEDGMENTS

The authors thank the patients and their families for allowing us to report their transplant outcomes as well as the entire staff of the Pediatric Blood and Marrow Transplant Program at Duke for providing excellent care to these families.

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

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