Growth, endocrine function and quality of life after haematopoietic stem cell transplantation

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DISTURBANCES OF GROWTH AND ENDOCRINE FUNCTION AFTER BUSULPHAN-BASED CONDITIONING FOR HAEMATOPOIETIC STEM CELL TRANSPLANTATION DURING INFANCY AND CHILDHOOD

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Abstract

It is generally assumed that busulphan/cyclophosphamide (Bu/Cy)-based conditioning regimens for haematopoietic stem cell transplantation (SCT) do not affect growth. We evaluated growth and endocrine function after Bu/Cy based conditioning in 64 children without a history of irradiation. Mean height standard deviation scores remained stable, but unexplained disturbances of growth after SCT were found in 17/48 (35%) of the children without growth-limiting disorders (10/23 in patients treated for haematological malignancies). In 10 patients, growth hormone (GH) secretion status was evaluated, and insufficient GH secretion was diagnosed in four patients. Thyroid function was evaluable in 52 patients. Two developed antibody-mediated thyroid disorders and 10 (19%) compensated primary hypothyroidism. Gonadal function was evaluable in 21 patients and was normal in all seven patients treated with low-dose Bu (8 mg/kg), whereas seven of the 14 children receiving high-dose Bu (16–20 mg/kg) developed gonadal failure; the majority of these patients had not been exposed to gonadotoxic therapy prior to Bu/Cy. Of the 49 evaluable patients, 16 developed subclinical hyperparathyroidism. We conclude that, besides gonadal and thyroid dysfunction, impaired growth and hyperparathyroidism often occur after Bu/Cy conditioning for SCT and that growth impairment may be the result of insufficient GH secretion.

Introduction

Disturbances of growth and endocrine function are important side effects of total-body irradiation (TBI)-based conditioning regimens in children undergoing haematopoietic stem cell transplantation (SCT). In an attempt to reduce these side effects, radiation-free, myeloablative regimens containing high doses of busulphan (Bu) and cyclophosphamide (Cy) have gained increasing popularity in children under 3 years of age, but also in older children undergoing SCT for malignant as well as non-malignant disorders. In the majority of studies on growth after radiation-free conditioning for SCT, it was concluded that Bu/Cy does not affect longitudinal growth [1-5]. Confronted with several children exhibiting unexplained growth impairment and decreased growth hormone (GH) secretion, we decided to evaluate growth and endocrine function in our paediatric Bu/Cy population.

Patients and methods

Patient selection

We studied all patients with at least 2 years of follow-up after Bu/Cy-based conditioning regimens for SCT, who did not have a history of irradiation (n=84). Before further analysis, we excluded patients with conditions related to multiple endocrine problems, that is, iron overload in patients treated for thalassaemia (n=14) and Blackfan–Diamond anaemia (n=4), as well as patients with Down syndrome (n=2). Characteristics of the remaining 64 patients are summarised in table 1. Patients were divided into three groups based on the indication for SCT: group I consisted of patients with a haematological malignancy (n=26), group II of patients with an immunodeficiency syndrome (n=22) and group III of patients with other inborn errors or aplastic anaemia (n=16).

The median age at the time of SCT was 2.3 years (range 0.1–14.7 years), the median time since SCT was 6.0 years (range 2.4–16.7 years). SCT was of allogeneic origin in all but one patient, who received an autologous SCT for acute myeloid leukaemia (AML).

Table 1. Patient characteristics

Indication SCT	Nr. of patients	Sex m:f	Age SCT (years) median (range)	Follow-up (years) median (range)
Group I: haematological malignancies	26	14:12	2.4 (0.5 – 12.4)	5.0 (2.4 – 11.3)
myelodysplastic syndrome (MDS)	13			
acute myeloid leukaemia (AML)	5			
acute lymphoblastic leukaemia (ALL)	5			
acute undifferentiated leukaemia (AUL)	1			
chronic myeloid leukaemia (CML)	1			
non-Hodgkin Lymphoma (NHL)	1			
Group II: Immunodeficiency syndromes	22	12:10	0.8 (0.1 – 11.3)	10.0 (3.8 – 16.7)
severe combined immunodeficiency (SCID)	13			
combined immunodeficiency (CID)	7			
Wiskott-Aldrich syndrome (WAS)	2			
Group III: inborn errors and aplastic anaemia	16	10:6	5.1 (0.7 – 14.7)	4.7 (3.0 – 9.9)
severe aplastic anaemia (SAA)	2			
X-linked adrenoleukodystrophy (X-ALD)	4			
haemophagocytic lymphohistiocytosis (HLH)	4			
metachromatic leukodystrophy (MLD)	2			
autosomal recessive osteopetrosis (OP)	2			
mucopolysaccharidosis type I/VI (MPS)	2			

Conditioning for SCT

Bu was given in 16 doses divided over 4 consecutive days in all patients, either orally (until 2000) or intravenously (i.v.) (from 2000 onward). The cumulative Bu dose was 8 mg/kg in 10 severe combined immunodeficiency (SCID) patients, 12–14 mg/kg in three myelodysplastic syndrome (MDS) patients and 16–20 mg/kg in the remaining 51 patients. The standard dose of cyclophosphamide was 50 mg/kg, once daily i.v. for 4 consecutive days (total dose 200 mg/kg). In three patients, receiving an SCT from a matched unrelated donor for storage diseases, a higher dose (60 mg/kg) was given (total dose 240 mg/kg). In all, 13 patients (12 MDS and one severe aplastic anaemia (SAA)) received a lower total dose of Cy (120 mg/kg, given as 60 mg/kg once daily for 2 consecutive days) in combination with a single gift of melphalan 140 mg/m^2 i.v.

Data collection

Data on height, pubertal development and endocrine function were collected from the clinical records. Height was expressed as standard deviation scores (SDS) for sex and age based on Dutch references [6]. Target height (TH) was calculated from parental height with correction for sex differences (13 cm) and secular trend (+4.5cm) [6]. TH range was defined as TH SDS ± 1.3 SD.

Peak stimulated GH secretion was measured after stimulation with clonidine (150 mg/m^2 p.o.) and, in case of an inadequate GH response to clonidine, also after stimulation with L-arginine (0.5g/kg i.v). To prevent false-negative GH responses to these stimulation tests, peri-pubertal children (girls >8.0 years of age and boys >10.0 years of age) were primed with sex steroids. Spontaneous GH secretion was measured in 36 samples, obtained by continuous blood withdrawal sampled at 20-min intervals between 20:00 and 18:00. Serum samples were analysed (in duplicate) with a time-resolved Immunofluorometric assay (Wallac, Turku, Finland; specific for the 22-kDa GH isoform, minimal detection limit 0.01 mg/l). Nocturnal spontaneous GH secretion was analysed with the Cluster pulse detection algorithm. Assay-specific reference values for 12-h nocturnal GH profiles (see table 3) were constructed from raw data obtained from 76 healthy prepubertal children [7] (median age 10.7, median height –1.1 s.d.) after correction for differences in GH assays [8] (courtesy of Professor K Albertsson-Wikland and Dr C Löfqvist, Sweden). Insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3) were analysed with specific radio immunoassays in the Wilhelmina Children's Hospital, Utrecht, the Netherlands, and expressed as SDS for age and gender [9].

Serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), free thyroxine (free T4), thyroid-stimulating hormone (TSH), parathyroid hormone (PTH), alkaline phosphatase (AP) and 25(OH) vitamin D3 were analysed with commercially available assays and interpreted using assay-specific references.

Data analysis and statistics

S-PLUS 6 Professional release 2 (Insightful Corp, Seattle, WA, USA) was used for statistical analysis, with the significance level (α) set at 5%. Changes in height SDS after SCT were analysed using a linear mixed-effect model with data grouped by individual patients, height SDS as dependent variable and time since SCT as independent variable with both fixed and random effects.

Possible effects of the variables ‘age at SCT’, ‘gender’ and ‘indication group’ on height SDS (intercept) and change in height SDS with time since SCT (slope) were analysed. Height measurements obtained after the onset of GH therapy were excluded from the analysis. In addition to the group analysis, the growth of individual patients was analysed. Growth was considered normal in patients with height SDS above -2 SD and height within TH range and no or only limited (<0.5 SD) decrease in height SDS. As an exception to these three criteria, a decrease in height SDS >0.5 SD towards TH SDS during the first 3 years of life was considered normal. Also, growth was considered normal if height SDS increased after SCT (catch-up growth), even if height SDS was below –2 SD or below TH range. Gonadal dysfunction was defined as elevated serum levels of FSH and/or LH. In boys, elevation of LH (with or without decreased levels of testosterone) was used as an indicator of Leydig cell failure, whereas elevation of FSH was used as an indicator of Sertoli cell failure (i.e. inability to produce enough inhibin B to suppress FSH secretion as a result of either Sertoli cell loss or loss of germ cells).

Results

Growth

Data on height development were missing in two patients from group II: a girl with combined immunodeficiency (CID) and kyphomelic dysplasia and a boy with Wiskott–Aldrich syndrome (WAS) living abroad (the referring centre stated that he was ‘growing well’). Of the remaining 62 patients, 571 height measurements were available. Height SDS at SCT and height SDS at the last visit are represented for the three subgroups in figure 1a.

A linear mixed-effect model did not show any change in height SDS with time since SCT: the estimated change in height SDS for the complete study population was 0.0001 SD/year. We then added the covariates ‘group’ (I, II or III), ‘age at SCT’ and ‘gender’ to the model (as single covariate and in different combinations). Of the covariates, only the factor ‘group’ had a significant effect: group II had a lower intercept (estimated height SDS at SCT) and a positive slope (estimated increase in height SDS with time since SCT 0.049 SD/year, P=0.038).

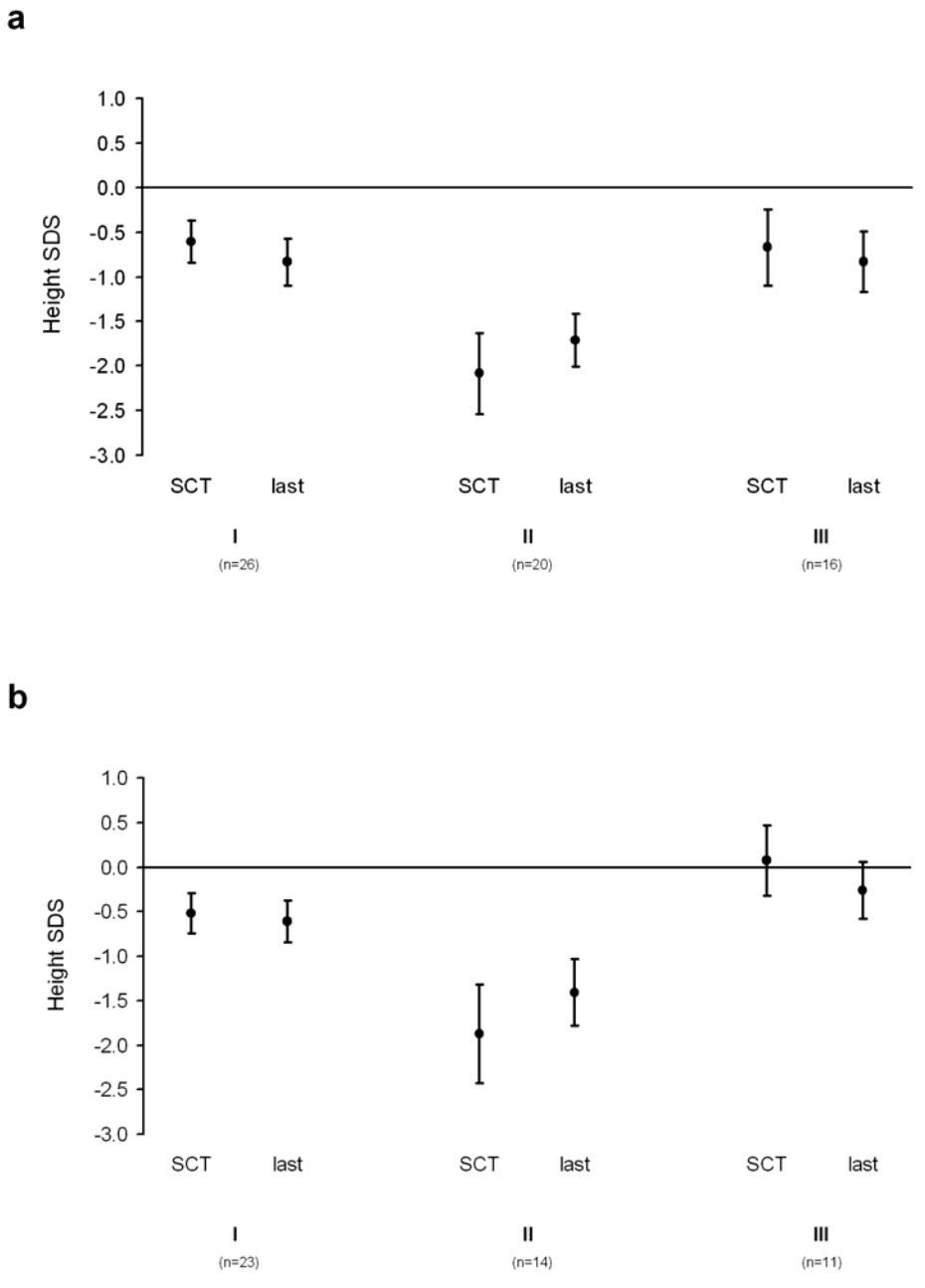

Figure 1. Changes in height SDS after SCT. The mean height SDS in the three subgroups at SCT and last visit. (a) Represents all patients (n=62). (b) Represents patients without growth-limiting disorders (n=48). Error bars represent standard error of the mean.

Separate analysis of the three subgroups yielded similar results, but the estimated increase in height SDS after SCT in group II (0.059 SD/year) was no longer significant (P=0.057). The fitted models (no covariates used) are summarised in table 2.

Table 2. Results of the linear mixed-effect models for changes in height SDS with time since SCT (SD/yr) in different groups of patients.

	Group	n	HSDS at SCT (95% CI)	Change after SCT (95% CI)
All patients		62	-1.15 (-1.55 to –0.75)	0.000 (-0.042 to +0.042)
	I	26	-0.61 (-1.08 to –0.13)	-0.044 (-0.131 to +0.043)
	II	20	-2.14 (-2.84 to –1.44)	+0.059 (-0.002 to +0.121)
	III	16	-0.76 (-1.58 to +0.06)	-0.035 (-0.114 to + 0.044)
No growth-limiting disorders		48	-0.77 (-1.19 to –0.35)	+0.002 (-0.045 to +0.049)
	I	23	-0.50 (-0.97 to –0.04)	0.000 (-0.073 to +0.073)
	II	14	-1.81 (-2.73 to –0.88)	+0.059 (-0.027 to +0.146)
	II	11	+0.01 (-0.69 to +0.71)	-0.074 (-0.156 to +0.008)

Data represent estimations and 95% confidence intervals (95% CI) of intercept (height SDS at SCT) and slope (change in height SDS with time). Data of all patients and of the patients without known growth-limiting disorders are presented.

Eight patients had a genetic disorder associated with impaired growth and/or skeletal abnormalities: two mucopolysaccharidosis (MPS), two osteopetrosis (OP), two Nezelof syndrome, one Noonan syndrome and one deletion of the adenosine deaminase gene. In addition, six patients had acquired growth-limiting disorders (three chronic graft-versus-host disease (GVHD), one chemotherapy-induced renal Fanconi syndrome, one treatment with high-dose steroids for immune-mediated pancytopenia and one malabsorption syndrome). None of the patients had a decrease in height SDS (>0.5 SD) due to a delayed onset of puberty.

Exclusion of the 14 patients with growth-limiting disorders from the analysis resulted in a higher intercept of the linear model (i.e. higher HSDS at SCT), but it had no effect on changes in height SDS after SCT (in group III exclusion even caused a tendency towards a decrease in height SDS in time, an effect caused by catch-up growth after SCT in the excluded patients with MPS and OP). Height SDS at SCT and height SDS at the last visit for the 48 patients without growth-limiting disorders are represented in figure 1b for the three subgroups.

Finally, we evaluated the growth pattern of individual patients and found unexplained abnormal growth in 17/48 patients (35%) without growth-limiting disorders (10/23 in group I, 3/14 in group II and 4/11 in group III). Of these 17 patients, 11 had a decrease in height SDS >0.5 SD (in six height SDS was also outside their TH range and/or below – 2 SD). Of the remaining six patients without decrease in height SDS >0.5 SD, height SDS was below TH range as well as below –2 SD. In the other three patients impaired growth was only based on height SDS below the TH range (one from each group, only the one from group II had a height <1 SDS). Total dose of Bu was 16–20 mg/kg in all 17 patients.

GH secretion status

GH secretion status was evaluated in 10 of the 17 patients with unexplained growth delay. The other seven patients either declined analysis of GH secretion or had a bone age that was too high to expect a significant effect of GH therapy on height (severe GH deficiency (GHD), warranting GH replacement in adult life was not suspected in any of these patients). Results of the evaluation of GH secretion are represented in table 3.

GH secretion was normal in 6/10 patients. In one patient, GHD was diagnosed, as both spontaneous and stimulated GH secretion were impaired. In two other patients spontaneous GH secretion was decreased (integrated mean GH and maximum GH below the 5th percentile for pre-pubertal children), but the GH response to pharmacological provocation was preserved, consistent with GH neurosecretory dysfunction (GHNSD) [10]. In addition, one patient who had already entered puberty by the time GH secretion was evaluated (no. 351, table 3) was also considered to have GHNSD, as all parameters of spontaneous GH secretion were near the lower limit of the reference interval

for pre-pubertal children (whereas spontaneous GH secretion is expected to increase after the onset of puberty). In addition, his serum levels of IGF-1 and IGFBP-3 were below –4 SDS.

Table 3. Evaluation of GH secretion in 10 patients with unexplained growth delay after SCT.

Patient					Spontaneous GH secretion			Stimulated GH secretion		IGF-1	BP-3
UPN	Disease	Bu (mg/kg)	Age (year)	Height (SD)	Mean GH (mU/L)	Nr of peaks	Max. GH (mU/L)	Test 1 (mU/L)	Test 2 (mU/L)	SDS	SDS
222	AML	20	6.6	-2.7	1.52*	4	9.2*	37.0	n.a.	-2.7*	-1.4
351	CID	16	14.3	-3.8	3.84	3	19.7	19.2*	28.7	-5.5*	-4.6*
Auto12	AML	20	9.2	-3.0	0.59*	1*	5.0*	6.5*	31.7	-1.1	1.2
318	ALL	20	8.8	-0.4	0.78*	4	5.3*	6.9*	5.6*	0.0	2.5
457	MDS	14	7.1	-1.1	5.40	5	20.7	26.1	n.a.	-2.7*	-0.1
364	AML	20	8.0	0.0	4.03	5	15.4	14.9*	13.7*	0.3	-1.4
414	FHL	20	9.3	-1.6	4.90	4	20.7	27.8	n.a.	-1.3	0.6
379	AML	20	6.6	-0.7	7.8	5	44.0	43.9	n.a.	0.8	-0.1
241	CID	20	12.0	-3.9	n.a.	n.a.	n.a.	27.9	45.0	n.a.	n.a.
228	SCID	18	9.0	-1.4	n.a.	n.a.	n.a.	59.3	n.a.	n.a.	n.a.
Reference:				Median (p5 – p95)	5.8 (3.2 - 20.6)	5 (3 - 7)	27.2 (13.1 – 94.9)				

UPN=unique patient number; *=decreased; n.a. = not available. References for spontaneous GH secretion are for prepubertal children (see methods).

All children with insufficient GH secretion were offered treatment with recombinant human GH (0.33 mg/kg/week once daily subcutaneous). One patient has refused treatment, another recently started therapy and two (nos. 351 and 222, see table 3) have received GH for the last 2 and 4 years, respectively. GH treatment resulted in a progressive increase in height SDS. At the last visit height had increased 1.3 SD since the onset of GH therapy in both patients

Thyroid function

TSH and free T4 were measured at regular intervals in 52 of the 64 patients. In 40 of these patients (77%) thyroid function was normal at all times. Two patients developed an antibody-mediated thyroiditis: hyperthyroidism in one boy, 4.5 years after SCT for X-linked adrenoleucodystrophy (X-ALD), and hypothyroidism in a girl, one year after SCT for acute lymphoblastic leukaemia (ALL) (this girl also developed an immune-mediated pancytopenia and was treated for 6 months with high doses of corticosteroids). Neither patient suffered from chronic GVHD. The remaining 10 children (six boys and four girls, mean age at the time of SCT 3.6 years; seven from group I, one from group II and two from group III) had one or more episodes of compensated hypothyroidism (i.e. mildly elevated serum TSH with normal serum free T4). None of the 10 children had chronic GVHD, and only one was diagnosed with acute GVHD. All patients with thyroid dysfunction had received 16–20 mg/kg busulphan; three patients had received melphalan as well. In three of the patients compensated hypothyroidism persisted. A TRH test showed primary hypothyroidism and all three are receiving thyroxin substitution.

Pubertal development and gonadal function

Owing to the young age of our population, the evaluation of puberty and gonadal function was only possible for 26 of the 64 patients. Data on pubertal development and gonadal function were incomplete in five patients (one in group I, four in group II). Seven patients (all SCID, three girls, age at last visit 11.7–16.5 years) had received a low cumulative busulphan dose of 8 mg/kg. Their gonadal function remained normal throughout follow-up. The other 14 patients had received 16–20 mg/kg busulphan. Data on their gonadal function are summarised in table 4.

Pubertal development was normal in all boys, and none developed Leydig cell failure. Sertoli cell failure developed in all three boys treated during puberty, none of them had received gonadotoxic chemotherapy prior to conditioning. Of the boys receiving SCT before the onset of puberty, none developed Sertoli cell failure, but 4/5 have only recently entered puberty. Both girls receiving SCT during puberty developed gonadal failure, none of them had received gonadotoxic chemotherapy prior to SCT. In addition, two of the four girls receiving SCT before the onset of puberty did not have a spontaneous onset of puberty and developed gonadal failure. One of them had been exposed to

gonadotoxic chemotherapy prior to conditioning. Until now, gonadal function seems to be preserved in the other two girls.

Table 4. Gonadal function after high-dose Bu/Cy in 14 patients

UPN	Gender	Age at SCT	Age at onset puberty	Age at last visit	Indicatio n SCT	Gonadotoxic treatment prior to conditioning	Bu/Cy (mg/kg)	Gonad al failure
227	m	0.6	10.0	10.5	OP	no	20 / 200	no ?
290	m	5.2	12.1	12.6	FHL	no	16 / 200	no ?
241	m	5.9	12.9	14.8	CID	no	20 / 200	no ?
317	m	6.1	10.9	11.5	X-ALD	no	20 / 200	no ?
245	m	10.7	unknown	19.8	WAS	no	16 / 200	no
358	m	12.4	pre SCT	17.2	MDS	no	16/120/mel	yes
357	m	13.2	pre SCT	17.5	SAA	no	16/120/mel	yes
432	m	14.0	pre SCT	16.2	X-ALD	no	16 / 240	yes
222	f	2.1	10.9	12.2	AML	yes	20 / 200	no ?
313	f	4.9	not yet induced	11.5	MLD	no	16 / 200	yes
105	f	7.9	11.0	16.1	CID	no	16 / 200	no
411	f	10.2	12.1 (induced)	14.2	MDS	yes	16/120/mel	yes
386	f	11.2	pre SCT	15.5	SAA	no	16 / 200	yes
406	f	14.6	pre SCT	18.3	MLD	no	16 / 200	yes

m=male; f=female; pre SCT=in puberty at time of SCT; mel=melphalan 140 mg/m^2; ?=less than 2 years in puberty; ages are in years; abbreviations of the indication for SCT are explained in table 1.

Calcium metabolism

Calcium metabolism was monitored in 49 patients using serum levels of PTH, AP and 25-OH-Vitamin D. Disturbances in calcium metabolism were encountered in 17 patients (35%). One patient, treated for autosomal recessive SCID (Bu/Cy 8/200 mg/kg), had developed renal Fanconi syndrome during the transplantation procedure. In another patient, vitamin D deficiency was identified as a probable cause of hyperparathyroidism, and treatment with vitamin D resulted in normalization of PTH. One patient had hypocalcaemia (serum calcium 2.15 mmol/l) as a possible cause of hyperparathyroidism, but although treatment with calcium and vitamin D resulted in normalization of serum calcium, PTH remained mildly elevated (6.0–7.5pmol/l). The remaining 14 patients with sub-clinical hyperparathyroidism (PTH 6.0–21.1 pmol/l) had

normal levels of both calcium (median 2.33 mmol/l; range 2.22– 2.55) and vitamin D (median 25-OH-Vitamin D 60 nmol/l; range 33–83; reference 30–120). In five patients, PTH was elevated despite a relatively high serum Ca >2.45mmol/l. Six of the 14 patients with hyperparathyroidism were treated with calcium tablets and vitamin D3, the others were advised to increase the dietary intake of calcium. In two patients treated with D3 and calcium (including the patient who initially had hypocalcaemia), PTH levels remained elevated despite therapy.

Other endocrine functions

Six patients had non transplant-related disturbances of adrenal function (four patients with X-ALD and two patients treated with high doses of corticosteroids). In the other patients adrenal function was normal.

Overall, disturbances of growth and/or endocrine functions were encountered in as many as 46 patients (72%), with more than one function disturbed in 17 (26%), even though the follow-up was not always complete for all endocrine organs, and gonadal function could not be evaluated in the majority of the patients due to the pre-pubertal age.

Discussion

The majority of studies have failed to show a negative effect of radiation-free, Bu/Cy-based conditioning on growth [1-5], and it is suggested that inadequate growth after SCT cannot be attributed to the Bu/Cy conditioning [5]. In our study, we did not find significant changes in height SDS either, but we did encounter unexplained impaired growth or GH secretion in as many as 35% of the patients who did not suffer from growth-limiting disorders (malnutrition, chronic GVHD, use of steroids, disorders of puberty, hypothyroidism, renal failure and gastrointestinal diseases were excluded). We believe that differences in patient population (i.e. inclusion of groups II and III) do not explain the differences in results between previous studies and the present one, as we found unexplained abnormal growth in 39% of the patients treated for a haematological malignancy (group I). A more likely explanation for the discrepancy is that in the other studies changes in height SDS were investigated in groups of patients only, whereas we also considered absolute

height SDS and evaluated individual growth patterns. In group analysis, impaired growth of one individual can be compensated by catch-up growth in another individual. In addition, results can be biased by differences in duration and intensity of follow-up, which we avoided by using mixed-effect model analysis with grouping of data from individual patients.

The ultimate end point of studies on growth is final height, but data on final height after Bu/Cy-based conditioning are very limited. In a multi-centre study from the EBMT on final height after SCT [4] no effect of Bu/Cy on final height could be detected. This study, however, included only 10 patients who had received Bu/Cy conditioning at a relatively high mean age of 11.7 years, and the authors concluded that the number of patients was too small to draw unequivocal conclusions. Compared to this EBMT study, the children included in the present study were much younger at the time of SCT, which may have contributed the differences in the effect of Bu/Cy on growth. Unfortunately, the young age at the time of SCT also resulted in the lack of data on final height in the present study.

In 10 of our patients, the impaired growth prompted us to evaluate GH secretion status, revealing decreased GH secretion in four of them (one GHD, three GHNSD). To our knowledge, GHNSD has never been described in Bu/ Cy-conditioned patients without a history of irradiation, whereas GHD has only been described once in two such patients [1]. From studies in children treated with cranial irradiation, it is known that physiological tests of GH secretion are more sensitive than pharmacological tests in diagnosing disturbances in GH secretion [11;12]. The continuing increase in height SDS in both patients treated with recombinant GH suggests that impaired growth resulted from GHNSD and emphasizes the relevance of physiological tests for GH secretion.

We found disturbances in thyroid function in 23% of Bu/ Cy-conditioned patients. Thyroid dysfunction is a well-known complication in survivors of SCT, and is found after TBI as well as radiation-free conditioning [13]. The most common disorders after radiation-free conditioning are euthyroid sick syndrome (ETS, diagnosed in up to 48% of patients) [14] and (compensated) hypothyroidism. ETS is most often detected in the first 3–12 months after SCT and is often related to complications, including GVHD [15]. In our clinic, thyroid function is not routinely screened until 12 months after SCT, and tri-iodothyronine (T3) is only measured on indication (e.g. hypothyroidism). Therefore, we have no information on the incidence of ETS in our population.

The incidence of (compensated) hypothyroidism in our population (19%) is slightly higher than the 10–15% reported in most studies of patients receiving radiation-free conditioning for SCT [5;14-17]. Of these studies, only Afify et al. [5] dealt exclusively with children (SCT for AML at a median age of 10.9 years) who had received Bu/ Cy-based conditioning, of whom 3/21 (14%) developed hypothyroidism. It is possible that the young age at the time of SCT is responsible for the slightly higher incidence in the present study, as the thyroid gland is believed to be more vulnerable in young children.

Several mechanisms for the development of hypothyroidism have been suggested. One possibility is that it is a direct (toxic) effect of chemotherapy on the thyroid gland. If so, the conditioning alone is capable of causing damage, as three of the 10 patients with hypothyroidism were not exposed to chemotherapy prior to SCT. Another possibility is that it is part of a GVHD-like phenomenon, or that chemotherapy induces mild thyroid damage that allows immune mechanisms to intervene, resulting in antibody-mediated thyroid disease [16]. Indeed, Kami et al. [15] found a correlation between acute GVHD and hypothyroidism after SCT, and they noticed antibody-mediated hyperthyroidism in eight patients (14%), seven of whom later developed compensated hypothyroidism. In our population, two patients (4%) developed an antibody-mediated disorder of the thyroid gland, but neither had manifestations of chronic GVHD. Thyroid-specific antibodies were absent in the other 10 patients, making an antibody-mediated thyroid disorder less likely as the cause of compensated hypothyroidism.

Gonadal damage is a well-known side effect of alkylating agents, such as busulphan, cyclophosphamide and melphalan [5]. The risk of gonadal failure increases with the cumulative doses of gonadotoxic therapies (e.g. radiation and/or alkylating agents), as well as with age at the time of therapy in females [18;19] (probably due to decreased number of primordial follicles). As far as we know, the only other data on gonadal function after high-dose busulphan (16–20 mg/kg) and Cy (200 mg/kg) in children who have not been exposed to gonadotoxic therapy prior to conditioning are reported by Couto-Silva et al. [20], who described gonadal failure in 2/3 girls who had not received gonadotoxic chemotherapy prior to conditioning. Several other reports concern patients with thalassaemia, who are at risk of gonadal failure due to iron overload as well [21;22]. Sanders et al. [22] reported that conditioning with Cy (200 mg/kg) did not result in gonadal failure in 24 girls and 27 boys receiving SCT for SAA before

puberty. Accordingly, in the present study, gonadal failure did not occur with the addition of a relatively low dose of busulphan (8 mg/kg) to such conditioning in patients with SCID (none of these patients had received gonadotoxic therapy prior to conditioning). In contrast, gonadal damage occurred in 3/8 boys and 3/4 girls who received 16–20 mg/kg busulphan and high-dose Cy without prior exposure to gonadotoxic agents. We therefore conclude that high-dose Bu/Cy itself is a major cause of gonadal damage, even in the absence of gonadotoxic therapy prior to conditioning. In addition, four of the patients who did not develop gonadal failure only recently entered puberty, warranting caution with the conclusion of normal gonadal function in these children. In girls with preservation of gonadal function, the risk of premature ovarian failure is likely to be increased [23]. Recovery of gonadal function, although rare, has been documented in both sexes [22]. Patients should be aware of the possibility of premature ovarian failure as well as recovery of gonadal function, in view of contraceptive measures and family planning.

Hyperparathyroidism was found in 35% of our evaluable patients. Probable causes were renal Fanconi syndrome in one patient and vitamin D deficiency in another. In the remaining 15 patients, hyperparathyroidism occurred despite adequate serum levels of calcium and vitamin D. Insufficient intake of calcium is a likely explanation for the elevated levels of PTH, but primary (i.e. calcium-independent) hyperparathyroidism cannot be excluded, especially since hyperparathyroidism was present in five patients despite a relatively high serum level of calcium and, in addition, suppletion of vitamin D and calcium did not result in normalization of PTH in two of the seven patients. Damage to the parathyroid glands can result in primary hyperparathyroidism, as is shown in patients who have received neck irradiation [24]. We speculate that high-dose alkylating agents may have similar effects on parathyroid glands, but more studies are needed to clarify both the origin and relevance of sub-clinical hyperparathyroidism in these patients. In view of the high incidence of sub-clinical hyperparathyroidism in our population, we suggest that the evaluation of calcium metabolism should be incorporated into follow-up protocols after SCT, and if necessary, suppletion of calcium and vitamin D should be started.

In conclusion, our data show that disturbances of both growth and endocrine functions are common after radiation- free Bu/Cy-based conditioning for SCT, even in the absence of chemotherapy prior to conditioning. We also show that impaired growth can be the result of insufficient GH secretion in these patients.

In addition, these data show that conditioning with high-dose Bu/Cy can result in gonadal failure in children without prior exposure to gonadotoxic chemotherapy, and that both hypothyroidism ad hyperparathyroidism frequently occur after radiation-free Bu/Cy-based conditioning for SCT. Therefore, both growth and endocrine function should be closely monitored in these patients. If growth is impaired without apparent explanation, GH secretion should be investigated, preferably by physiological testing.

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