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

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Vormgeving: B. Bakker

Drukker: Ponsen & Looijen b.v., Wageningen

ISBN-10: 90-9020336-2

ISBN-13: 978-90-9020336-2

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PROEFSCHRIFT

ter verkrijging van

de graad van Doctor aan de Universiteit Leiden,

op gezag van de Rector Magnificus Dr. D.D. Breimer,

hoogleraar in de faculteit der Wiskunde en

Natuurwetenschappen en die der Geneeskunde,

volgens besluit van het College voor Promoties

te verdedigen op donderdag 27 april 2006

klokke 16:15 uur

door

BOUDEWIJN BAKKER

geboren te Dubbeldam

in 1967

PROMOTIECOMMISSIE

Promotores: Prof. dr. J.M. Wit

Em. prof. dr. J.M. Vossen

Co-promotor: Dr. W. Oostdijk

Referent: Prof. dr. H.A. Delemarre-van de Waal, Vrije Universiteit Amsterdam

Overige leden: Prof. dr. S.L.S. Drop, Erasmus Universiteit Rotterdam

Prof. dr. R.M. Egeler

Prof. dr. J.A. Romijn

The Research presented in this thesis was financially supported by the Netherlands' Orginazation for Scientific Research (NWO / ZonMw).

For publication of this thesis, financial support from Ferring Geneesmiddelen B.V., Ipsen Farmaceutica B.V., Novo Nordisk Farma B.V., Pfizer B.V., Eli Lilly Nederland B.V. and ZonMw is gratefully acknowledged.

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General introduction

Chapter 1

Introduction

Over the last three decades, haematopoietic stem cell transplantation (HCT) has become an important treatment modality for a wide range of life-threatening haematological and immunological disorders in both children and adults, with over 25,000 transplants in Europe in the year 2003 [1]. With an increasing number of long-term survivors, the long-term consequences of HCT become increasingly important. Late effects may result from the disease for which HCT is performed (including its initial treatment), from toxicity of the conditioning regimens, and (in allogeneic HCT) from chronic graft-versus-host-disease (cGVHD).

Chronic GVHD

cGVHD is the prime cause of transplant related mortality and contributes both directly and indirectly to many late complications [2;3]. The incidence of cGVHD is increasing due to the increase of alternative donors (e.g. haplo-identical family members and matched unrelated donors), alternative sources of haematopoietic stem cells (peripheral blood stem cells instead of bone marrow), and use of donor lymphocyte infusions for treatment of relapse or prophylaxis to prevent relapse in patients at high risk for relapse of their malignancy [4]. Although a wide range of organs can be affected by cGVHD (e.g. skin, hair, nails, eyes, mouth, gastro-intestinal tract, liver and respiratory tract), endocrine organs are usually not directly affected. Treatment of GVHD with high doses of glucocorticosteroids, however, will have its impact on growth, adrenal function and bone mineral density.

Conditioning regimens

Successful engraftment of allogeneic haematopoietic stem cells will not occur in the presence of a competent immune system. Therefore, most recipients will have to be 'conditioned' for HCT. This conditioning aims at induction of space for progenitor cells to engraft and immunosuppression to accept the allogeneic graft [5]. It may be myeloablative (i.e. eradicating the host’s haematopoietic

system), or immunoablative (i.e. only suppressing the host's immune alloreactivity, nowadays called 'reduced intensity conditioning'). It is effectuated by high doses of chemotherapy, often combined with total-body irradiation (TBI) and sometimes with anti-T-cell antibodies. In the first decade of HCT, most myeloablative conditioning regimens consisted of single-fraction TBI and high dose cyclophosphamide (120 mg/kg). In an attempt to reduce late effects from radiation-induced toxicity, most centres have replaced single fraction TBI (radiation dose 7-10 Gy) by fractionated TBI (total radiation dose 10-16 Gy, fraction size 1.2-3.0 Gy, fraction interval 6-24 hours). In addition, radiation-free conditioning regimens were introduced, containing high doses of busulphan (16-20 mg/kg) or treosulphan (30-42 mg/m^2), combined with cyclophosphamide (120-200 mg/kg), or Melphalan (140 mg/m^2). Sometimes other chemotherapeutic agents are added for an additional anti-leukaemic effect (e.g. cytosine-arabinoside or etoposide).

Chemotherapy and late effects after HCT

Most late effects of chemotherapeutic agents used in conditioning regimens result from alkylating agents, such as cyclophosphamide (Cy) and busulphan (Bu). The most important late effect of high doses of these agents is gonadal damage, contributing to azoospermia in boys and premature ovarian failure in girls. In addition, alkylating agents may cause lung damage, resulting in interstitial pneumonitis and pulmonary fibrosis. Busulphan, used in radiation-free conditioning regimens, may give rise to cataract formation in some patients [6;7], although far less frequent than after TBI-based conditioning for HCT. All alkylating agents increase the risk of secondary tumours.

Radiotherapy and late effects after HCT

TBI is one of the most important causes of late effects after HCT. TBI contributes to non-endocrine late effects such as interstitial pneumonitis and pulmonary fibrosis, renal dysfunction, cataract, dental dysplasia, decreased salivary function, and secondary tumours [8;9]. Endocrine late effects of TBI include damage to the growth plate, growth hormone deficiency (GHD), gonadal failure, and primary hypothyroidism [2;10]. TBI increases the risk of secondary tumours, with a latency period of 10 years or more in most cases.

Radiobiology of TBI

In this section, the different types of radiation damage and the rationale behind fractionation are briefly discussed. Radiation damage can be divided in stochastic and non-stochastic effects. Stochastic effects are effects that occur on a random basis with the chance of occurrence (but not the severity) increasing with dose (e.g. secondary tumours). These effects typically have no threshold value. Non-stochastic effects, also called deterministic effects, are those in which the severity of the effect varies with the dose and for which a threshold value does exist. Deterministic effects of ionising radiation depend on total dose, fraction size, and fraction interval and dose rate (i.e. the radiation dose received in a given time).

The basis of fractionation can be explained by the five R's of radiotherapy:

- Radiosensitivity (different cell types have different radiosensitivity)
- Repair (cell types differ in their capacity for repair of sub-lethal radiation damage)
- Repopulation (between doses repopulation takes place)
- Redistribution (effects of radiation on individual cells depend on their position in the cell cycle; between fractions, cells are 'redistributed' among different phases of the cell cycle. Cells that were in a relatively radioresistant state at time of first exposure may have become more radiosensitive during subsequent exposure to radiation)
- Reoxygenation (in hypoxic state, cells are relatively radioresistant; reoxygenation of hypoxic tissues make cells more sensitive to subsequent doses of radiation)

Cell survival curves represent the relation between radiation dose and surviving fraction of cells. They are described by a combined linear and quadratic model, determined by radiation dose and the first two R's of radiotherapy: radiosensitivity (α, linear relation to dose) and capacity for cellular repair (β, quadratic relation to dose) [11].

$$S(D) = e^{-(\alpha D + \beta D^2)}$$

S(*D*): the fraction of cells surviving a dose *D*;
α: a constant describing the initial slope of the cell survival curve;
β: a smaller constant describing the quadratic component of cell killing.

Tissues that are relatively radioresistant and/or have a high capacity for cellular repair have a low α/β ratio, whereas those that are radiosensitive and/or have a low capacity of repair have a high α/β ratio. Fractionation of the total radiation dose in fractions of 1.2-3.0 Gy with an interval of at least 6 hours, will lead to much greater reduction of radiation damage in tissues with low α/β ratios compared to tissues with high α/β ratios. Therefore, if a tumour has a high α/β ratio, fractionation can result in reduction of radiation damage to normal tissues, which allows for higher total doses without increasing normal tissue damage [12]. Some haematological malignancies, however, are not very radiosensitive, resulting in reduced tumour kill if fractionation is applied. In a study by Cosset et al., fraction size sensitivity (i.e. influence of fraction size on tumour survival) was high in chronic myeloid leukaemia (CML), variable in acute lymphoblastic leukaemia (ALL) and low in acute non-lymphoblastic leukaemia (ANLL) [13]. Due to this diversity in radiosensitivity, one fractionation scheme (e.g. 6x2.0 Gy) will not fit all patients. This explains why outcomes from different TBI-schedules are similar in unselected patients populations due to diversity in radiosensitivity between the different tumours [14].
Besides reducing tumour kill in some tumours, fractionation reduces the immunosuppressive effects of TBI. Therefore, fractionation of total TBI dose is associated with a higher incidence in graft failure, especially in patients receiving T-cell depleted grafts. To reduce these negative effects of fractionation on tumour kill and immunosuppression, total dose is usually higher in fractionated TBI (12-15 Gy) compared to single fraction TBI (7-10 Gy). This increase in total TBI dose reduces the possible beneficial effects of fractionation. Two large single-centre, prospective randomised studies comparing 10 Gy single fraction TBI to fractionated TBI (12 or 14.85 Gy) did not find any difference in the main outcome parameters (i.e. overall survival, relapse-free survival and interstitial pneumonitis) [15;16]. The most common late

effects of TBI in children are gonadal failure and growth plate damage. As these organs have high α/β ratios, the benefit of fractionation is probably limited.

Endocrine late effects after HCT

Gonadal function, puberty and fertility

Of the hypothalamus-pituitary-gonadal axis, the gonads are the most sensitive to chemotherapy and radiation. Both radiation and alkylating agents may induce gonadal failure, busulphan being one of the most gonadotoxic chemotherapeutic agents.

In boys, the testicular germinal epithelium is much more vulnerable to both radiation and chemotherapy than Leydig cells are [17]. As a result, the vast majority of boys will have severely reduced fertility due to damage to the germinal epithelium, but their pubertal development is normal. On the other hand, recovery of spermatogenesis has been reported in a small number of patients after TBI [18-21].

After TBI-based conditioning for HCT in girls, the risk of ovarian failure increases with age at TBI as well as with time since TBI [22;23]. This relation between ovarian failure and age of TBI is less well-established after Bu/Cy based conditioning [10]. As a result of radiation damage to the uterus [24;25], pregnancies in women with a history of TBI are at high risk of complications. The combined results of two large studies report spontaneous abortion in 25%, preterm delivery in 53% and low birth weight (<2.5 kg) in 56% [19;21].

At the hypothalamic-pituitary level, radiation doses >18 Gy are required to induce precocious puberty (most often seen in girls) [26-28], and even higher doses (>24 Gy) are needed to induce hypogonadotrophic hypogonadism [29;30]. Therefore, precocious puberty and hypogonado-trophic hypogonadism are almost exclusively seen in patients who had received prophylactic cranial irradiation prior to HCT.

Thyroid function

Thyroid dysfunction is reported after TBI-based conditioning as well as after radiation free conditioning. Of the hypothalamic-pituitary-thyroid axis, the thyroid gland is by far the most sensitive to radiation and chemotherapy, and

therefore hypothyroidism will be the result of damage to the thyroid gland itself. In most cases a compensated primary hypothyroidism is seen, characterized by an increase of thyroid stimulating hormone (TSH) in combination with a normal serum free thyroxine (FT4) level. Overt hypothyroidism with decreased FT4 is rare. The incidence of thyroid dysfunction increases with 1) time since HCT, 2) younger ages at time of TBI and 3) increasing TBI doses. In addition, thyroid dysfunction appears to be more common after unfractionated TBI (up to 45%) compared to fractionated TBI (15%) [10].

Growth and growth hormone secretion

Impaired growth is an important complication of HCT, which occurs in the vast majority of children conditioned with TBI, but only rarely after conditioning with Bu-Cy. Major causes of impaired growth are chronic GVHD and its treatment with glucocorticosteroids, damage to the epiphyseal growth plate, impairment of GH secretion, hypothyroidism and hypogonadism. Growth impairment is most prominent during puberty, with a blunted pubertal growth spurt in most patients [31].

Other endocrine functions

The remaining endocrine tissues (e.g. adrenal glands, parathyroid glands, pancreas and adipose tissue) are relatively resistant to radiation and chemotherapy. Therefore, direct late effects of either radiation or chemotherapy on these organs are exceptional.

Outline of this thesis

Chapter 2 describes the late effects of TBI as a single toxic agent in rhesus monkeys on growth, thyroid gland and pituitary gland. Anthropometrical data were collected; thyroid and pituitary glands were examined; serum levels of thyroid stimulating hormone, free thyroxin, insulin-like growth factor-I and its binding protein-3 were measured. In humans, the exact role of TBI is difficult to assess due to the superimposed and/or synergistic effects of other factors present in clinical settings (e.g. cytostatics, antimicrobial drugs, GVHD, steroids and the underlying disease itself). The rhesus monkeys in this study received

TBI as single toxic agent and therefore provide a unique opportunity to study effects of TBI.

Chapter 3 is a report on the effect of irradiation on longitudinal growth, growth plate architecture and the expression of parathyroid hormone related peptide (PTHrP) and Indian hedgehog (IHh) in tibial growth plates of rats. PTHrP and IHh are key regulators of pace and synchrony of chondrocyte differentiation, and irradiation will disturb both these processes.

In **chapter 4** results of a cross-sectional study on both endocrine and non-endocrine late effects of HCT during childhood are presented in a population of adult survivors of childhood HCT. Effects on growth, gonadal function, thyroid function, bone mineral density, lung function, renal function, eyes and skin were evaluated.

Chapter 5 reports the results of the largest multi-centre study on factors that play a role in the final height outcome of patients who underwent HCT during childhood. The study includes data on 181 patients with aplastic anaemia, leukaemia, and lymphoma who had HCT before puberty and who had reached final height.

In **chapter 6** pubertal development and growth after TBI-based conditioning for allogeneic HCT for haematological malignancies is described in children who received HCT in our centre.

Chapter 7 describes patterns of growth and body proportions in children receiving TBI for HCT in our centre before onset of puberty. It is one of the largest single-centre studies on growth and final height after TBI-based conditioning for HCT, and the first study to analyse the effects of unfractionated TBI on body proportions in boys and girls separately, thereby identifying sex-differences in development of body proportions after single dose TBI. For each sex, a model of growth after TBI is presented, in which effects of time since HCT, age at HCT, and puberty are the major determinants of changes in height SDS.

In **chapter 8** results of a study on growth hormone secretion and the effects of GH therapy on growth after TBI-based conditioning for HCT in our centre are reported. In the study, the model for growth after TBI (as described in chapter 7) is used to estimate the net effect of GH therapy. The study is one of the few studies on the effect of GH therapy after TBI that report final height, and the only study in which most children treated with GH were not considered GH deficient.

Chapter 9 describes the effects of radiation-free Bu/Cy based conditioning on growth and endocrine function in children without a history of irradiation. In contrast to most other reports, individual growth curves were analysed in addition to group analyses, and impaired growth was encountered in a significant proportion of patients.
In **chapter 10** Quality of life is investigated in adult survivors of childhood HCT, using both generic and disease-specific questionnaires. In addition coping strategies are investigated.
Finally, the results and implications of the different studies are discussed in **chapter 11** and summarised in **chapter 12**, followed by a summary in Dutch in **chapter 13**.

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EFFECTS OF TOTAL-BODY IRRADIATION ON GROWTH, THYROID AND PITUITARY GLAND IN RHESUS MONKEYS

Radiotherapy and Oncology 1999;51:187-192

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Abstract

Aim: To investigate the effect of total-body irradiation (TBI) on growth, thyroid and pituitary gland in primates.

Materials and methods: Thirty-seven rhesus monkeys (mean age 3.1 ± 0.6 years) received either a low-dose (4 - 6 Gy) TBI (n = 26) or high-dose (7 - 12 Gy) TBI (n = 11) and were sacrificed together with 8 age-matched controls after a post-irradiation interval of 5.9 ± 1.5 years. Anthropometric data were collected; thyroid and pituitary glands were examined; serum levels of thyroid stimulating hormone (TSH), free thyroxin (FT4), insulin-like growth factor-I (IGF-1) and its binding protein-3 (IGFBP-3) were measured.

Results: Decrease in final height due to irradiation could not be demonstrated. There was a dose-dependent decrease in body weight, ponderal index, skinfold thickness and thyroid weight. The latter was not accompanied by elevation of TSH or decrease in FT4. Structural changes in the thyroid gland were found in 50% of the irradiated animals. Levels of IGF-I and IGFBP-3 did not differ between the dose groups, but the high-dose group had a lower IGF-I/IGFBP-3 ratio.

Conclusions: Total body irradiation had a negative effect on body fat. There was no evidence of (compensated) hypothyroidism, but dose-dependent decrease in thyroid weight and changes in follicular structure suggest some effect of TBI on the thyroid gland. The decreased IGFI/IGFBP-3 ratio in the high-dose group can indicate that the somatotrophic axis was mildly affected by TBI. These results show that TBI can have an effect on the physical build and thyroid gland of primates even in the absence of cytostatic agents or immunosuppressive drugs.

Introduction

Total-body irradiation (TBI) is frequently used in combination with high-dose chemotherapy in conditioning regimens for haematopoietic stem cell transplantation (HCT). Unfortunately this aggressive conditioning has negative effects on several organs including those of the endocrine system [1]. Examples of negative effects on endocrine organs are decreased fertility and hypogonadism, (compensated) hypothyroidism, and growth hormone deficiency (GHD), respectively [2]. In children, TBI and HCT often result in impaired growth and reduction of final height [3;4]. Although TBI is considered to be an important etiologic factor in these disorders, its exact role is difficult to assess due to the superimposed and/or synergistic effects of other factors used in clinical settings (e.g. cytostatics, antimicrobial drugs, GVHD, corticosteroids and the underlying disease itself). Therefore, studies in animals without (previous treatment of) an underlying disease are helpful in the investigation of TBI as a single toxic factor. Radiation experiments in primates are of relevance since the response to radiation in monkeys does not seem to be significantly different from that in man. This has been demonstrated for acute effects on the haematopoietic system [5] and late effects such as tumour induction [6]. Furthermore an outbred species such as the rhesus monkey (Macaca mulatta) is more representative as an animal model to assess the effect of TBI as a single toxic factor than are inbred rodents. Results of the effect of TBI, without interference of other medication such as cytostatics or immuno-suppressive drugs, on the eye [7] and on hepatic and renal function [8] are already available. This article describes the effect of TBI as a single toxic factor on both the thyroid gland and somatotrophic axis.

Materials and methods

Animal population

Between 1963 and 1989 approximately 100 rhesus monkeys received TBI in the Biomedical Primate Research Centre (BPRC) of Rijswijk, the Netherlands, in experiments on both the efficacy of the HCT procedure and the late effects of TBI. In a terminal experiment 37 irradiated animals and 20 control animals

were sacrificed. Anthropometric measurements were taken and after anaesthesia (using ketamin and vetrancyl) the animals were heparinised and euthanised. Tissue samples gathered during autopsy were distributed to several research institutes interested in late effects of TBI. We were able to obtain blood samples and tissue samples of the thyroid and pituitary gland. The mean age of the irradiated animals was 8.9 ± 1.6 years (range 6.2 - 11.8). There were eight age-matched control animals with a mean age of 8.9 ± 2.0 years (range 6.8 - 12.5). The mean age of the control animals that were excluded from the analyses was 24.9 ± 5.8 years (range 17.8 - 34.8). Total body irradiation was given at a mean age of 3.1 ± 0.6 years (range 2.0 - 4.6), which means that most of the animals had just entered puberty at the time of the TBI. Age at time of irradiation did not differ between the low-dose group (LD) and the high-dose group (HD). The post-irradiation interval (overall 5.9 ± 1.5 years) was longer in the high-dose group compared to the low-dose group: median 6.8 (4.8 - 8.3) years versus 5.7 (3.3 - 7.4) years (P = 0.003). All animals involved in the present study were bred within the BPRC colony and kept under identical housing conditions. They were fed commercial food pellets (Hopefarms) and a diet of fresh fruit and vegetables. The animals were procured, maintained and used in accordance with Dutch law and regulations, the Animal Care and Use Committee and the Animal Ethical Committee approved all experiments.

Irradiation and additional treatments

The total-body irradiations were performed at an instantaneous dose-rate of 0.3 Gy min^{-1} with either 300 kV or 6 MV X-rays. Monkeys were irradiated in a specially designed cage that was slowly rotated along its longitudinal axis in the beam in order to obtain an optimal dose distribution over the animal. At a later stage the animals were irradiated bilaterally. Radiation doses received by the monkeys are expressed as absorbed dose in soft tissue averaged over the animal [9]. Three groups of animals were distinguished: control animals (n = 8), animals that received a relatively low TBI dose (4 - 6 Gy as a single fraction; n = 26), and those which received a high TBI dose (7 - 8.5 Gy as a single fraction; n = 8, or 12 Gy in two single fractions on two consecutive days; n = 3). The animal characteristics in the different dose groups are presented in table 1. Both the TBI doses and dose rate correspond well with those used in our clinic in several hundred patients transplanted at our centre since 1965. Patients

transplanted for severe aplastic anaemia, immune-deficiency syndromes or haemoglobinopathies received 4 - 5 Gy single fraction TBI. Patients with haematological malignancies receive either 7 - 8 Gy as a single fraction, or 12 Gy in two single fractions on two consecutive days (instantaneous dose rates used are always 0.25 ± 0.05 Gy min^{-1}).

Table 1. Number of male and female monkeys in subsequent dose categories

TBI dose	Males	Females
Control animals (n=8)	2	6
Low-dose TBI (n=26)		
4.0 Gy	3	-
5.0 - 5.3 Gy	15	5
6.0 Gy	3	-
High-dose TBI (n=11)		
7.0 Gy	1	-
8.0 Gy	2	1
8.5 Gy	3	1
2 x 6.0 Gy	2	1

After TBI seven animals (all LD) received supportive care only, the other animals received additional treatment to enhance the recovery of bone marrow. Additional treatment consisted of either cytokines only (n = 18, one HD), or HCT (n = 5, all HD), or both HCT and cytokines (n = 7, five HD). Cytokines used were human granulocyte macrophage colony-stimulating factor (GM-CSF), rhesus monkey interleukin-3 (IL-3) or rhesus monkey interleukin-6 (IL-6) for approximately 14 days.

Morphological, functional and histological assessments

Anthropometrical measurements consisted of body weight, subscapular skinfold thickness, upper leg length and lower leg length, sitting height and

head circumference. All measurements were done according to standard protocols[10]. Total length was estimated by adding sitting height, upper leg length and lower leg length. Ponderal index was calculated as (body weight x 100) / sitting height[11]. Thyroid status was evaluated by determining thyroid weight, serum levels of free thyroxin (FT4) and thyroid stimulating hormone (TSH). Both FT4 and TSH were assayed at the Leiden University Medical Centre; FT4 was measured by radio-immunoassay (RIA) and TSH by immuno-radiometric assay (IRMA), (both from DPC, Los Angeles CA). The thyroid glands of 26 irradiated (seven high-dose TBI) and eight control animals were sectioned and stained at the Veterinary Faculty of Utrecht University with haematoxylin-eosin (HE), periodic-acid-Schiff (PAS), and immunohistochemical staining using antibodies against calcitonin. The somatotrophic axis was evaluated by measuring serum levels of insulin-like growth factor-1 (IGF-I) and its binding protein 3 (IGFBP-3) (assayed at the Wilhelmina Children's Hospital, Utrecht[12]). Sections of the pituitary glands were stained with HE, PAS, orange-G and immunohistochemical staining using antibodies against growth hormone.

Statistical analyses

Results are expressed as mean (SD) or median (range) as indicated. The results of the different dose groups were compared by non-parametric tests (Mann-Whitney U test and Kruskal-Wallis H test). Dose dependency was analysed by Spearman's correlation for both sexes separately. Linear regression analysis with calculation of partial correlation coefficients was done on the total group of all animals controlling for possible confounding factors such as sex, age and body weight. All analyses were performed using two-sided tests.

Results

Anthropometric measurements

Because of the differences between both sexes, separate analyses were done for each sex. Table 2 shows the results of the anthropometric measurements.

Table 2. Auxological results in the different dose groups. Data presented as mean (SD).

	MALES				FEMALES			
	Reference	Control (n=2)	Low-dose (n=21)	High-dose (n=8)	Reference	Control (n=6)	Low-dose (n=5)	High-dose (n=3)
Body weight (kg)*	10.8	7.8 (0.5)	7.0 (1.2)	5.9 (0.8)	8.7	4.9 (0.8)	4.5 (0.7)	3.8 (0.5)
Ponderal index**	19.0	14.0 (1.6)	12.9 (1.7)	11.3 (1.3)	16.8	10.2 (1.0)	9.3 (1.1)	8.0 (0.7)
Skinfold (cm)*	4.1	4.0 (1.4)	3.5 (1.4)	2.6 (0.8)	5.4	4.3 (0.8)	2.5 (0.7)	2.3 (1.2)
Sitting height (cm)*	56.8	56.1 (2.8)	53.8 (3.0)	52.4 (2.3)	51.9	47.5 (3.5)	48.2 (1.4)	47.3 (2.6)
Upper leg length (cm)	n.a.	18.5 (2.3)	18.5 (1.3)	18.1 (0.9)	n.a.	15.0 (1.0)	15.5 (0.6)	15.9 (1.2)
Lower leg length (cm)	n.a.	21.8 (0.4)	21.0 (1.5)	20.6 (1.5)	n.a.	17.9 (0.7)	18.1 (0.8)	19.3 (1.3)
Head circumference (cm)	n.a.	29.5 (0.1)	30.1 (1.9)	28.9 (1.7)	n.a.	26.4 (2.0)	26.8 (0.8)	26.4 (1.3)
Estimated height (cm)	n.a.	96.3 (0.1)	93.3 (5.1)	91.1 (3.1)	n.a.	80.4 (4.0)	81.7 (2.2)	82.5 (4.7)

* Reference values are means, based on data from Schwartz and Kemnitz[13] on 6-14 year old animals.

** Calculated from mean values of body weight and sitting height derived from data of Schwartz and Kemnitz[13].

n.a. Not available.

Comparison of the results for the different dose groups revealed a decrease with increasing TBI doses in body weight in males P = 0.036), a decrease in ponderal index in both males (P = 0.045) and females (P = 0.033), and a decrease in subscapular skinfold thickness in females (P = 0.018). Radiation-dose dependency was confirmed by correlation analyses for body weight (males: r = -0.37; P = 0.042 and females: r = -0.57; P = 0.033), for ponderal index (males: r = -0.37; P = 0.039 and females: r = -0.69; P = 0.006), and for skinfold thickness in females (r = -0.65; P = 0.013). After controlling for the effects of sex and age, the partial correlation coefficient between body weight and TBI dose was r = -0.51 (P = 0.001), between ponderal index and TBI dose r = -0.55 (P < 0.001) and between skinfold thickness and TBI dose r = -0.39 (P = 0.011). The other parameters, including sitting height and estimated total length did not show significant differences between the dose groups, nor were there indications for radiation-dose dependency of those parameters.

Functional and morphological evaluation of the thyroid gland

The mean thyroid weight decreased with increasing TBI dose (table 3; $P < 0.001$). There was a negative correlation between thyroid weight and TBI dose ($r = -0.59$; $P < 0.001$), which remained after controlling for body weight (partial $r = -0.51$; $P = 0.001$). Free T4 and TSH levels did not differ between the various dose groups. Table 3 (upper part) summarises the results of the thyroid evaluation.

Table 3. Results of thyroid and somatotrophic evaluation. Organ weights as mean (SD); serum parameters as median (range).

	Control	Low-dose TBI	High-dose TBI
Weight thyroid (g)	0.59 (0.16)	0.47 (0.16)	0.30 (0.10)
TSH (mIU/L)	0.14 (0.01-2.41)	0.35 (0.37-1.72)	0.42 (0.12-2.44)
Free T4 (pmol/L)	4.2 (1.8-13.1)	6.9 (2.2-18.1)	5.8 (3.3-10.0)
Weight pituitary (g)	0.096 (0.024)	0.084 (0.018)	0.082 (0.017)
IGF-1 (mg/L)	0.37 (0.23-0.62)	0.40 (0.21-0.88)	0.32 (0.22-0.57)
IGFBP3 (mg/L)	1.55 (1.01-3.74)	1.88 (1.17-2.44)	1.71 (1.30-2.03)
IGF-1/IGFBP3 ratio	0.19 (0.16-0.34)	0.21 (0.12-0.47)	0.17 (0.12-0.23)

On histological examination 13 of the 26 irradiated monkeys (six out of seven in the high-dose group and seven out of 19 in the low-dose group) had small follicles (which were lined with higher cuboidal epithelium) compared to the non-irradiated animals. An example of these differences is shown in figure 1.
Animals with these structural changes had a lower thyroid weight compared to animals without these changes (mean: 0.522 versus 0.354 g; $P = 0.004$) and there was a correlation between TBI dose and the presence of structural changes ($r = 0.54$, $P = 0.001$). One irradiated monkey (TBI dose 5.0 Gy) had a macrofollicular goitre with focally distinct papillary hyperplasia. The papillae and some newly formed follicles had high columnar cells and many resorption vacuoles were present. The distended follicles had flat epithelial cells. No other histological abnormalities were found in the thyroid sections.

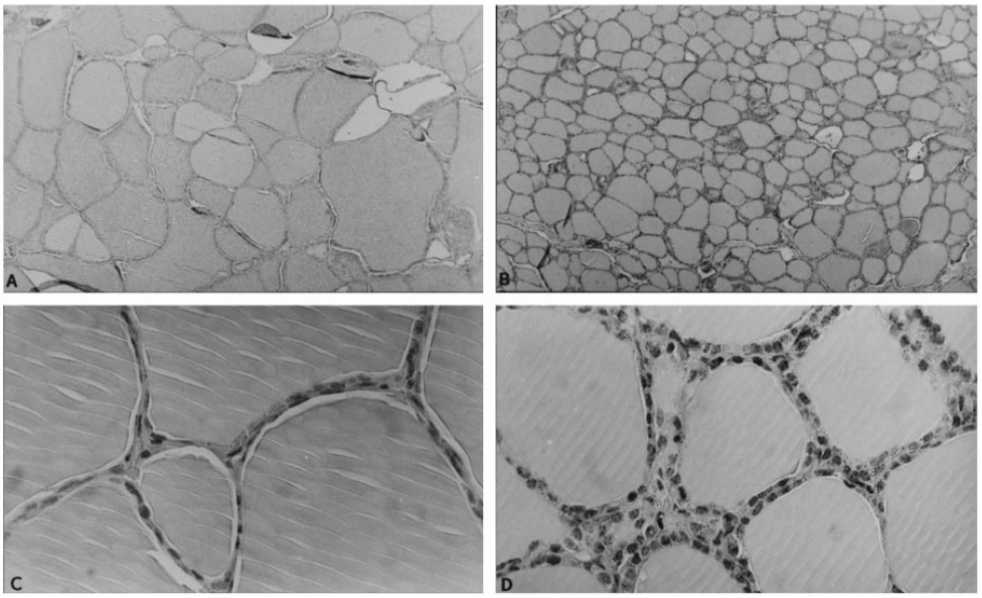

Figure 1. Example of the typical thyroid abnormalities found in irradiated monkeys (left side) compared to the normal thyroid tissue of a non-irradiated monkey (right side). Note the decrease in follicle size lined with higher cuboidal epithelium in the irradiated animal (staining: Haematoxylin-Eosin; magnification: a & b: 10x5; c & d: 10x40).

Functional and morphological evaluation of the somatotrophic axis

Results of evaluation of the somatotrophic axis and the pituitary gland are included in table 3 (lower part). Pituitary weight did not differ between the various dose groups. No correlation was found between TBI dose and pituitary weight even after controlling for body weight and sex. The plasma levels of IGF-I and IGFBP-3 did not differ between different dose groups. However, the ratio of IGF-I and IGFBP-3 was higher in the low-dose group compared to the high-dose group ($P = 0.047$). On histological examination no abnormalities were found in the pituitary glands apart from an area with hyperplasia of prolactin-producing cells in one irradiated monkey (TBI dose 8.5 Gy).

Discussion

Studies on the effect of TBI in HCT patients are complicated by confounding variables such as the initial disease, the use of cytostatics in both initial

treatment and conditioning regimens, the complications of allogeneic marrow transplantation and the effects caused by post-HCT medication (e.g. corticosteroids, cyclosporine A and antibiotics). The animals in this study therefore offer a unique possibility to investigate the effects of TBI as a single toxic agent in primates. Compared to the measurement data published by other authors[11;13] the animals used as controls in this study had a relatively low body weight and ponderal index, and the females had low sitting heights and subscapular skinfold thickness. In spite of this, though, body weight, ponderal index and subscapular skinfold thickness were clearly influenced by TBI: the high-dose irradiated animals were skinny compared to age-matched non-irradiated animals. A possible explanation for these changes are provided by Griffiths et al.[14]. They describe alterations in gastrointestinal regulatory peptides in the irradiated monkeys, which could have resulted in anorexia. In contrast to the observations in children after TBI and HCT, no effect of TBI on growth could be found in the present study. The loss of height potential in children is most prominent during puberty, as many patients have an impaired pubertal growth spurt[3;15]. Rhesus monkeys exhibit only a small pubertal growth spurt compared to humans[16], and therefore, impairment of this growth spurt would not have major effects on final height. Sonneveld and van Bekkum[17], however, showed that TBI (as single toxic agent) can cause inhibition of growth in rhesus monkeys from the same colony as the animals in the present study. Radiation doses of 7.5 Gy or higher were required and the effect was more pronounced in animals irradiated before the age of 40 months. The relatively low-doses used in most animals in our study and the relatively high ages at the time of irradiation could explain the lack of growth impairment (at the age of three the animals have attained approximately 90% of their adult sitting height)[10]. The difference in thyroid weight between the various dose-groups and the negative correlation between thyroid weight and TBI dose suggest an effect of TBI on the thyroid gland. Small follicles lined with higher epithelium, as found in thyroid glands of irradiated animals, are usually associated with a high activity of the follicular epithelial cells. We speculate that, in some animals, radiation has induced damage to thyrocytes which is compensated by their increased activity. This increased activity may have resulted in a decreased colloid content, which, in conjunction to a possible reduction in cell number, could explain the decrease in thyroid weight. In humans receiving a TBI and HCT for haematological malignancies, (compensated) hypothyroidism occurs

in 15 - 50% of the patients[18]. The incidence of radiation-induced (compensated) hypothyroidism depends on radiation dose, fractionation schedule and post-irradiation interval[19]. The small number of animals in the high-dose group and the limited post-irradiation interval could explain why we did not find hypothyroidism. The incidence of malignant tumours of the thyroid gland is increased after irradiation[20], and young patients with papillary thyroid carcinoma often have a history of irradiation of the thyroid gland[21;22]. After HCT and TBI, patients are at risk for secondary malignancies[23;24], and although most of those are of haematological origin[25;26] the incidence of thyroid carcinomas is also increased[23]. In this study no evidence for malignant thyroid tumours was found in any of the irradiated monkeys. This could, however, be caused by the relatively short post-irradiation interval, as radiation induced (thyroid) malignancies in monkeys (as in humans) generally occur after a latency period of ten years or more[6]. After irradiation of the hypothalamus-pituitary axis a decreased secretion of hormones from the anterior lobe of the pituitary can occur. In most cases growth hormone is the first of these hormones to be decreased in the circulation. Radiation induced growth hormone deficiency (GHD) is dose dependent and the incidence increases with increasing post-irradiation intervals[27;28]. The animals in the high-dose group were therefore more likely to suffer from GHD than those in the low-dose group. However, the diagnosis of GHD is difficult and requires stimulation tests or evaluation of GH secretion patterns by frequent sampling, which could not be performed in the present study. Growth hormone deficiency is reflected by decreased serum levels of IGF-1 and IGFBP-3[29]. Even in man, however, the normal ranges of IGF-I and IGFBP-3 are wide and distinction between values of normal and GH-deficient subjects is difficult. Information on IGF-1 and IGFBP-3 levels in serum of normal rhesus monkeys is scarce, and values show considerable variations: Schwartz and Kemnitz[13] describe mean IGF-I levels of approximately 100-350 ng/ml in young adult rhesus monkeys, whereas Liu et al.[30] mention mean IGF-I levels of 600-1600 ng/ml. We therefore depended on our control animals as reference for normal values of IGF-I and IGFBP-3. Although the absolute levels of IGF-I and IGFBP-3 were not different between the radiation groups, the lower IGF-1/IGFBP-3 ratio of the animals in the high-dose group suggests less circulating free IGF-I, which may indicate subtle changes in the somatotrophic axis by TBI. Histological examination did not reveal any changes in the pituitary gland; whether the hyperplasia of prolactin-

producing cells is related to the TBI is unknown. In summary, we demonstrated an effect of TBI on body weight, ponderal index and skinfold thickness, but not on height. The histological changes and the decrease of thyroid weight with increasing TBI doses are indications for radiation-induced thyroid damage and compensatory reactions. Although the changes in body composition and the normal IGF-I and IGFBP-3 levels do not suggest radiation-induced GHD, there was a decreased IGF-I/IGFBP-3 ratio in the high-dose group, which could indicate a subtle effect of TBI on the somatotrophic axis. We therefore conclude that TBI-doses of 4-12 Gy with an instantaneous dose rate of 0.3 Gy min^{-1} can have an effect on the thyroid gland and on the physical build of primates even in the absence of cytostatic agents or immunosuppressive drugs.

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Effect of X-irradiation on growth and the expression of parathyroid hormone-related peptide and Indian hedgehog in the tibial growth plate of the rat

Hormone Research 2003;59:35-41

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Abstract

Aim: To study the effect of irradiation on the longitudinal growth and the expression of parathyroid hormone-related peptide (PTHrP) and Indian hedgehog (IHh) in tibial growth plates of rats.

Materials and methods: At 3 weeks of age, 30 male rats received a single fraction of irradiation (8 Gy) to their right hind limb, and small groups of animals were sacrificed 1, 2, 3, 5, 7, 10, 15, and 26 weeks after irradiation. Weight and length of both irradiated and non-irradiated tibiae were measured, and sections of the tibiae were stained with HE. PTHrP and IHh were visualized using immunohistochemical techniques.

Results: Radiation resulted in persistent growth delay of the irradiated tibiae, with a difference in length of more than 10% between the irradiated and the non-irradiated tibiae 15 weeks or more after irradiation. The growth plate architecture was disturbed, and the expression of both PTHrP and IHh was decreased in the irradiated tibiae.

Conclusion: As PTHrP and IHh are key regulators of both the pace and the synchronisation of the differentiation of growth plate chondrocytes, the reduced expression of PTHrP and IHh may contribute to the changes found after irradiation.

Introduction

In children treated for cancer, radiation has a direct effect on the epiphyses which results in disruption of growth plate architecture and contributes to the impaired growth by a yet unknown mechanism [1;2].
Many tissues show changes in the expression of regulatory proteins in response to radiation damage, a phenomenon known as 'humoral radiopathology' [3]. These humoral factors are often growth factors or other mediators of cell proliferation and differentiation (e.g., transforming growth factors, fibroblast growth factors, tumour necrosis factor, and others). Although the effects of irradiation on growth and growth plate architecture are extensively studied for over 50 years [4-8], little is known about the effects of irradiation on the expression of growth factors involved in the regulation of chondrogenesis in the epiphyseal growth plate.
Parathyroid hormone-related peptide (PTHrP) and Indian hedgehog (Ihh) are paracrine/ autocrine factors that control the pace and synchrony of chondrocyte differentiation and are believed to co-ordinate the development of the growth plate and to influence growth rate [9].
As radiation affects architecture and growth rate of the growth plates, we were interested in its effect on the expression patterns of PTHrP and IHh. Therefore, we studied the effect of local irradiation on longitudinal growth and on the expression of both PTHrP and IHh in rat tibial growth plates.

Materials and Methods

All animal experiments were approved by a local ethical committee and performed according to Dutch law and regulation.

Irradiation

At the age of 3 weeks, 30 male Wistar rats received a single dose of X-irradiation (8.0 Gy) to the right hind limb (Philips X-ray generator, operating at 250 kV and 15 mA, equipped with a Thoraeus filter which resulted in a dose rate of 1.6 Gy/min). The left hind limb served as an internal control. Irradiation was performed at the Department of Clinical Oncology, using a setup that was

previously used for irradiation of rat gastrocnemius muscle. Details on the irradiation procedure are described in an earlier report by Hermens et al. [10]. The animals were anesthetised with a mixture of Aescoket® (50 mg/kg i.p.) and Rompun® (2 mg/kg i.p.) prior to irradiation. The right hind limbs were irradiated in posterior-anterior direction from the knee joint down. The rest of the body was protected with 2 mm thick lead plates. Special attention was given to the position of the testes, in order to prevent radiation damage. The focus–skin distance was 25 cm.

Animal Housing and Sample Collection

After irradiation, the animals were placed (2 per cage) in a light and temperature-controlled environment and were given standard laboratory chow and water ad libitum. At post-irradiation intervals of 1, 2, 3, 5, 7, and 10 weeks, groups of 4 animals were decapitated and both the irradiated and the non-irradiated tibiae were dissected and stripped. The same was done at 15 and 26 weeks with groups of 3 animals. Tibiae were weighed, and the tibial length was measured with a caliper. The tibiae were then split mid-saggittally in two equal halves and further processed for immunohistochemical analyses.

Immunohistochemistry

The detailed immunohistochemical procedures were previously described by Van der Eerden et al. [11]. The aspect of a growth plate section varies with the plane of the section (exactly craniocaudal or angulated) as well as with the position of the section (central or more peripheral in the growth plate). To ensure comparable sections, much effort is put on splitting the tibiae exactly mid-saggitally in equal halves and on the embedding and positioning of the samples on the microtome. Furthermore, only the first 15 sections of each sample were used to prevent the use of peripherally cut sections.

For PTHrP detection in the proximal tibial growth plates, the primary antibody was rabbit-derived polyclonal IgG raised against amino acids 34–53 of human PTHrP which is homologous to the PTHrP sequence in the rat (Oncogene Science, Cambridge, Mass., USA). For the detection of IHh, the primary antibody was goat-derived polyclonal IgG raised against the carboxy terminus of human IHh protein which cross-reacts with mouse and rat IHh (Santa Cruz Laboratories, Santa Cruz, Calif., USA). For optimal comparability, sections of

the irradiated and non-irradiated growth plates of animals of the same age were processed in the same experiment.

Measurements and Statistical Analyses

SPSS version 10.0 (SPSS, Chicago, Ill., USA) was used for statistical analyses. Differences in tibial length were analysed with regression models using a linear and 3rd-order curve fit. Histological measurements were done on digital micrographs of growth plate sections, using an image analysis program (Image-Pro Plus 3.0; MediaCybernetics, Silver Spring, Md., USA). We decided to use digital imaging, since blinding of the samples was not possible, due to the clearly visible differences between the irradiated and non-irradiated growth plates, making counting subjective.

In each growth plate, we measured the mean width of the individual growth plate zones, the mean height of individual columns in the proliferative zone, the amount of intervening matrix (as percentage of total growth plate area), the number of cells in the late proliferative and early hypertrophic zone, and the number of PTHrP-positive and IHh-positive cells in this 'transitional' zone. In individual animals, the results of the irradiated growth plate were compared to those of the normal growth plate. As there were only 3 or 4 animals at each time point, the animals were then clustered into three age groups: young (1–3 weeks after irradiation), middle-aged (5–10 weeks after irradiation), and old (15–26 weeks after irradiation). Differences between irradiated and non-irradiated growth plates were analysed in each age group using A Wilcoxon signed-rank test.

Results

There were no visible effects of the irradiation in any of the animals, i.e., we did not observe functional impairment or skin lesions of the irradiated hind limb. In all animals, the irradiated tibia was shorter as compared with the non-irradiated tibia. This was noticed already 1 week after irradiation. Furthermore, the difference increased with increasing post-irradiation intervals, suggesting continuous growth delay (figure 1a).

On histological examination, a clear disruption of the growth plate architecture was found at all times after irradiation (figure 2). The columns were less straight and less parallel to each other, and many columns did not extend

across the entire growth plate. The mean reduction in column height in the proliferative zone was 38% in the young animals (p = 0.002), 27% in the middle-aged animals (p = 0.04), and 13% in old animals (not significant). Furthermore, there were some clusters of cells that were not organised in columns, as well as clustered columns. The amount of intervening matrix was increased in the middle-aged (mean increase 26%; p = 0.03) and older animals (mean increase 17%; p = 0.04), but not in the younger animals. There were some cells with a hypertrophic appearance within the proliferative zone (see arrows in figures 2b and d) and some columns failed to complete the transformation from cartilage to bone, which resulted in cartilage islands within the trabecular bone (see arrowheads in figures 2f and 3f).

Due to these structural changes, the different zones (i.e., resting, proliferative, hypertrophic, and calcifying zones) were less well defined, making it difficult to establish the exact width of each zone. We could not establish significant changes in the growth plate width nor in the width of individual zones (data not shown).

Both the absolute and relative numbers of PTHrP-positive cells in the irradiated growth plates were reduced (figure 3a–f). This reduction was seen already 1 week after irradiation and did not restore with increasing time interval after irradiation. The reduction was seen in both the stem cell zone and (most prominent) in late proliferating and early hypertrophic chondrocytes which were previously shown to express PTHrP [11]. The mean relative reduction in PTHrP-positive cells in the 'transitional' zone was 52% in the young animals (p = 0.02), 43% in the middle-aged animals (p = 0.04), and 36% in the old animals (p = 0.05). We saw a similar reduction in the number of IHh-positive cells in the irradiated tibiae (figure 3g–h). Since overall staining of IHh was very weak, computer-aided digital imaging and quantification turned out to be unreliable.

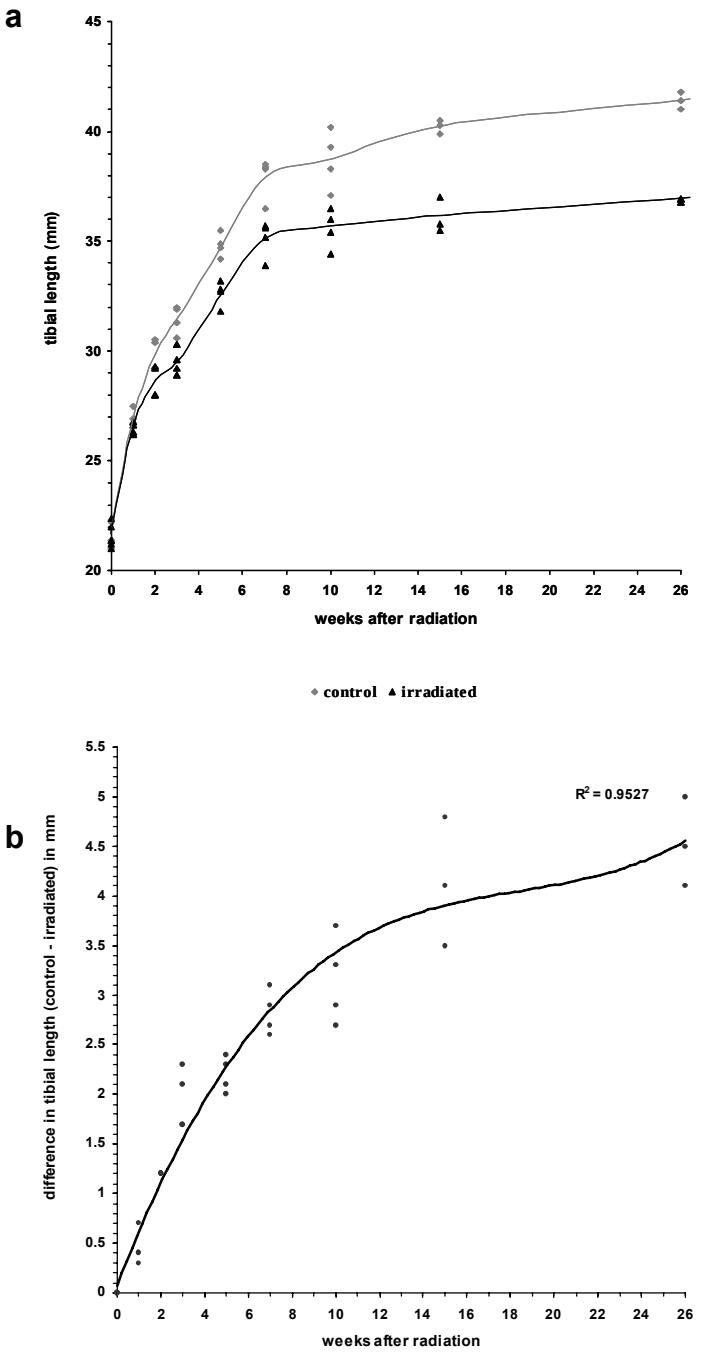

Figure 1. **a.** Tibial length of irradiated (▲) and non-irradiated (♦) legs 0 to 26 weeks after irradiation. **b.** Individual differences in tibial length (control minus irradiated tibia) versus weeks after irradiation.

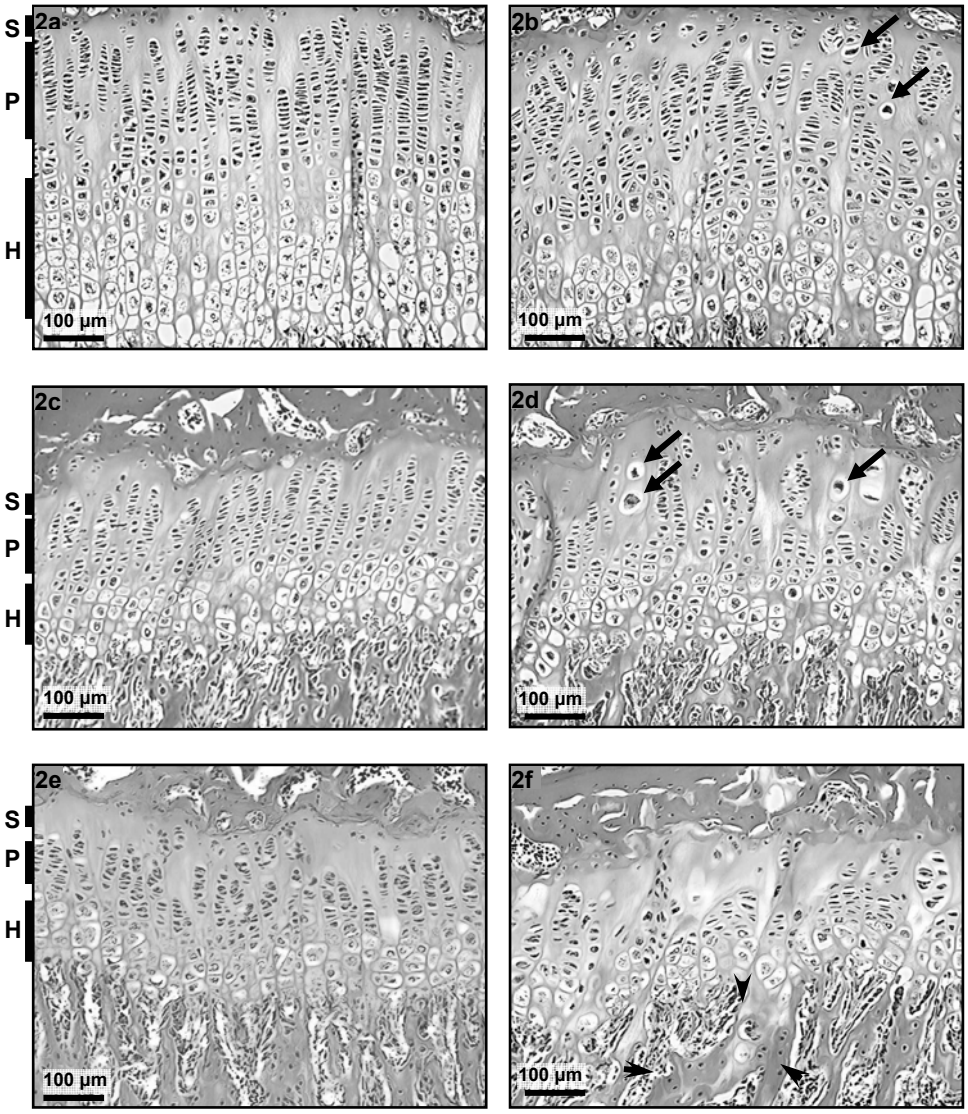

Figure 2. Morphological changes after irradiation.
Irradiated tibiae are presented on the right-hand side. HE staining. ***a,b*** 2 weeks after irradiation. ***c,d*** 7 weeks after irradiation. ***e,f*** 15 weeks after irradiation. Note the disorganisation of the growth plate and the presence of hypertrophic cells (arrows in b and d) in the proliferative zone and the cartilage islands in metaphyseal bone (arrowheads in f). S = Stem cell zone; P = zone of proliferation; H = zone of hypertrophy.

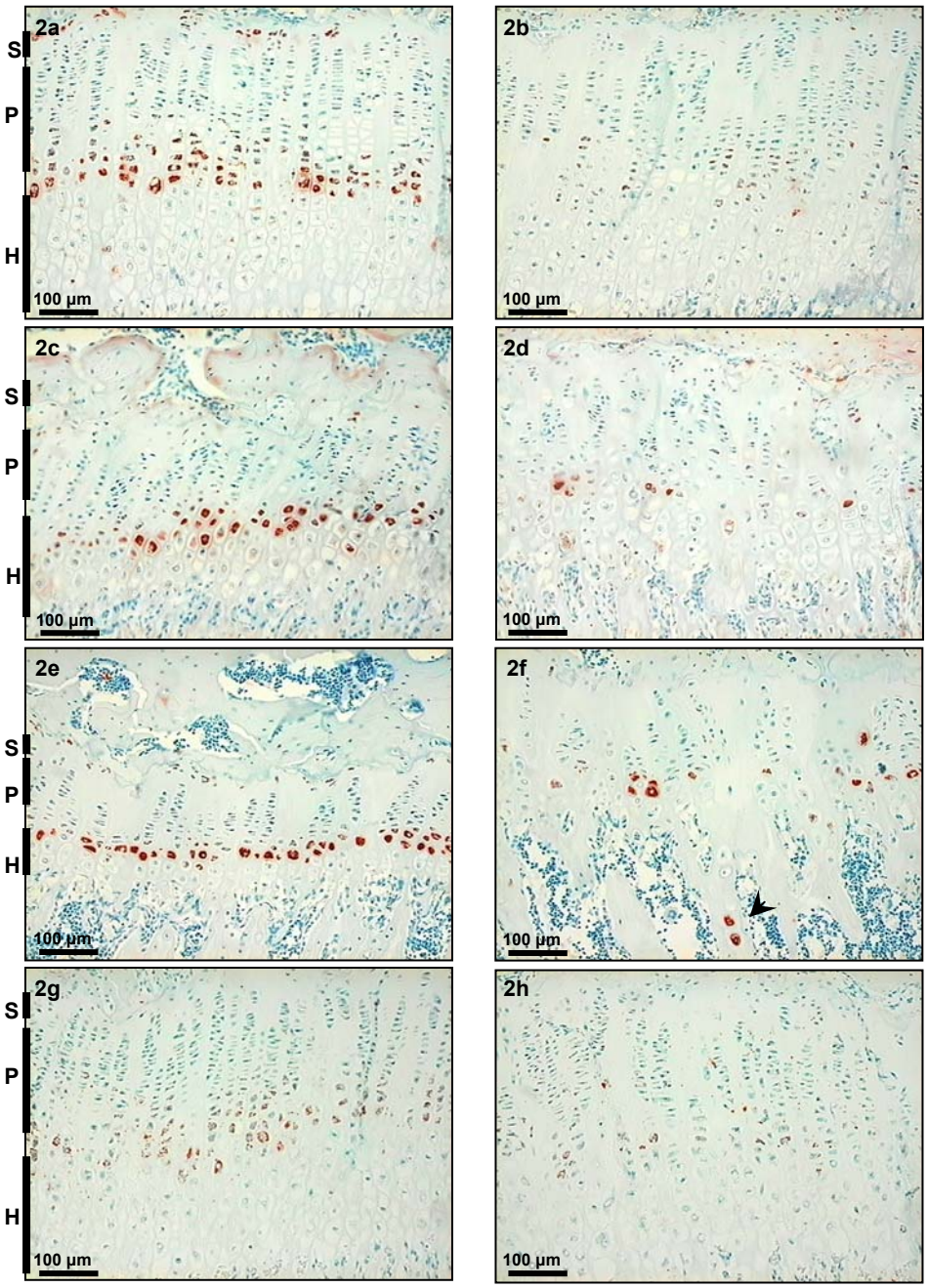

Figure 3. Presence of PTHrP and IHh in the tibial growth plates at different times after irradiation. Irradiated tibiae are presented on the right-hand side. PTHrP and IHh levels are clearly reduced after irradiation. **a,b** PTHrP 2 weeks after irradiation. **c,d** PTHrP 7 weeks after irradiation. **e,f** PTHrP 15 weeks after irradiation; note the islands of hypertrophic cells (with PTHrP staining) within the bone (arrowheads in f). **g,h** IHh 2 weeks after irradiation. **S** = Stem cell zone; **P** = zone of proliferation; **H** = zone of hypertrophy.

Discussion

In our clinic, many haematopoietic stem cell transplant recipients who are treated for haematological malignancies show growth delay after a single fraction of 8.0 Gy of total-body irradiation [12]. As this radiation dose is known to damage the growth plate architecture in many species, including rats [8;13], we decided to use this dose for our experiments. As could be expected, a single dose of 8.0 Gy to the right hind limb resulted in structural as well as functional damage (growth delay) to the epiphyseal growth plate. The difference in tibial length between the irradiated and non-irradiated limb increased during the whole follow-up period, suggesting that growth delay was persistent, and there was certainly no catch-up growth. This is in line with the human situation, where damage to the growth plates also results in persistent growth retardation.

The increase in intervening matrix in the middle-aged and old animals suggests that growth retardation could be the result of exhaustion of germinal cells after radiation-induced cell death in the stem cell zone. The decreases in PTHrP and IHh, however, suggest that changes in paracrine/autocrine factors could also contribute to both growth delay and structural changes. PTHrP is a paracrine/autocrine factor, produced in most cell types in the body. Its functions include the regulation of cell cycle, differentiation, apoptosis, and developmental events [14]. In prenatal growth plates, it delays the transition of chondrocytes from a proliferative towards a hypertrophic state and synchronises the rate of differentiation in the growth plate [15]. If PTHrP is over-expressed, the transition from proliferation to differentiation is impaired, resulting in prolonged proliferation and delayed differentiation [16]. In the absence of PTHrP, however, this transition is accelerated, which leads to premature differentiation and growth delay [17]. Therefore, both the PTHrP and the PTH/PTHrP-receptor-deficient mice show accelerated hypertrophy and mineralization in the cartilage. Furthermore, PTH/PTHrP receptor knockout mice show delayed vascular invasion, whereas the double homozygous PTHrP and PTH/PTHrP receptor knockout mice do not. Thus, PTHrP must slow vascular invasion by a mechanism independent of the PTH/PTHrP receptor [18]. PTHrP expression is stimulated by IHh which is expressed in early hypertrophic chondrocytes [19]. An increase in PTHrP slows down differentiation and results in a reduction of IHh-producing cells, thus forming a negative

feedback loop which co-ordinates the development of the growth plate and influences growth rate [20]. All components of this feedback loop (i.e., PTHrP, IHh, and their respective receptors) are also present in the post-natal growth plate of the rat [11], and the feedback loop is, therefore, supposed to be functional after birth as well.

A recently published in vitro study on irradiated avian growth plate chondrocytes [21] describes a dose-dependent decrease in both PTHrP mRNA and PTHrP protein (but not other autocrine and paracrine factors) 24 h after irradiation which was related to a radiation-induced increase in cytosolic calcium. In addition to these findings, we found in our in vivo experiments that PTHrP and IHh continue to be reduced after longer post-irradiation intervals (up to 26 weeks). Furthermore, radiation resulted in growth delay and disorganisation of the columnar structure of the growth plate, which could indicate impaired synchronisation of the processes of proliferation and differentiation in the growth plate. As PTHrP and IHh play a key regulatory role in these processes, it is not unlikely that the radiation-induced disturbances in growth plate differentiation are related to the changes in PTHrP and/or IHh expression we found after irradiation. In normal growth plates, however, premature differentiation mediated by a decrease in PTHrP expression would not only result in growth delay, but also in accelerated differentiation and increased bone formation, something we did not see in our experiment. This implies that differentiation is also impaired. Indeed, in vitro experiments in other species have shown a decrease in matrix production and mineralization after irradiation [22]. There are several possible explanations for the reduced expression of PTHrP we found after irradiation: (1) irradiation may have disturbed the proliferation of chondrocytes which forces them into differentiation, achieved by a reduction in PTHrP expression, and (2) irradiation has impaired the differentiation of chondrocytes, and the decrease in PTHrP expression is an attempt to overcome this. Both explanations imply that decreased PTHrP expression is a regulatory mechanism, but they do not explain why the decreased expression of PTHrP did not increase IHh expression, as could be expected, if the negative feedback loop is present in postnatal growth plates. A third possibility is that the reduction in the expression of both PTHrP and IHh is just a consequence of the impaired function of differentiating chondrocytes, as (at least in other species) other

differentiation markers are also reduced after irradiation (e.g., alkaline phosphatase and collagen X) [22].
Whatever the mechanism, however, a reduction in PTHrP and IHh is expected to have an effect on growth and differentiation. We, therefore, conclude that a reduced expression of both PTHrP and IHh may contribute to the disturbances in growth and growth plate architecture seen in rats after irradiation.

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LONG TERM CONSEQUENCES OF ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION DURING CHILDHOOD: RESULTS OF A CROSS-SECTIONAL SINGLE-CENTRE EVALUATION

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Introduction

Over the past 35 years, haematopoietic stem cell transplantation (HCT) has become an important treatment modality for a wide range of life-threatening haematological and immunological disorders in both children and adults. Successful engraftment of allogeneic haematopoietic stem cells, however, will not occur in the presence of a competent immune system. Therefore, most recipients will have to be 'conditioned' for HCT. Moreover, when HCT is done to cure a haematological malignancy, intensive conditioning is needed, eradicating most of the host's haematopoiesis. The latter conditioning is qualified as myeloablative and is mostly effectuated with high dose chemotherapy, often combined with total-body irradiation (TBI). Such aggressive conditioning regimens will have their impact on the integrity of many other tissues as well. With an increasing number of long-term survivors, the late effects of HCT have become perceptible more distinctly, and ways to prevent them more imperative.
As one of the first centres in the world to perform a successful allogeneic bone marrow transplantation [1], the Leiden University Medical Centre (LUMC) has a large experience in HCT in children. Unfortunately many of our patients from the early days of HCT were lost to follow-up after reaching adulthood. We therefore decided to trace and recall as many long-term survivors as possible and evaluate the late effects of HCT in these patients.

Patients and Methods

Patient selection

We included all patients who received a HCT between 1974 and 1995 for either a haematological malignancy or severe aplastic anaemia (SAA) before the age of 18 years, who had a disease-free survival of at least 5 years and were at least 16 years of age at the time of the study. Patients living abroad were excluded, as well as one patient who would be unable to participate due to severe impairment caused by a massive intra-cranial haemorrhage in the period of HCT. A total of 33 of the 49 patients responded to our invitation, of whom 22 gave their informed consent. The main reasons for not participating

were: patients attending another hospital for their regular check-ups, the time-consuming nature of the study, and the study being regarded as too physically and emotionally taxing. Patient characteristics are presented in table 1.

Table 1. Patient Characteristics

Patient nr.	Sex (m/f)	Indication for HCT	TBI dose (Gy)	Age HCT (years)	Age study (years)	Follow up (years)	Remarks
1	m	SAA	-	10.2	31.0	20.8	
2	m	SAA	-	12.4	31.6	19.2	
3	m	SAA	-	15.1	32.2	17.2	
4	f	SAA PNH	4.0	17.9	27.8	10.0	
5	m	ALL 1	6.0 x 2	13.9	21.1	7.2	
6	f	ALL 1	6.0 x 2	15.7	21.8	6.0	
7	m	ALL 2	7.5	4.1	18.4	14.4	Cranial irradiation before HCT
8	m	ALL 2	7.5	5.8	19.6	13.8	Cranial irradiation before HCT
9	f	ALL 2	6.0 x 2	11.9	18.1	6.1	
10	f	ALL 2	8.0	14.3	29.3	15.1	Cranial irradiation before HCT
11	f	JMML	5.0	0.9	19.2	18.4	
12	f	AML	7.5	7.0	25.8	18.8	Papillary thyroid carcinoma
13	m	AML	7.5	7.4	21.6	14.3	
14	f	AML	8.0	10.7	24.8	14.1	
15	f	AML	8.0	10.1	24.3	14.2	
16	f	AML	8.0	14.3	30.3	16.0	2 x spontaneous abortion
17	f	AML	8.0	15.9	25.2	9.3	
18	f	AML	8.0	15.2	31.3	16.1	
19	m	AML	8.0	10.3	21.1	10.8	HCT twice, progressive lung disease, avascular hip necrosis
20	m	MDS	7.5	9.6	23.5	13.9	
21	f	CML (Ph+)	7.5	10.0	27.6	17.8	Avascular hip necrosis
22	m	NHL 2	6.0 x 2	14.1	20.7	6.6	

SAA	Severe Aplastic Anaemia
SAA PNH	Severe Aplastic Anaemia Paroxysmal Nocturnal Haemoglobinuria
ALL1	Acute Lymphoblastic Leukaemia in 1st remission
ALL 2	Acute Lymphoblastic Leukaemia in 2nd remission
JMML	Juvenile MyeloMonocytic Leukaemia
AML	Acute Myelogenous Leukaemia in 1st remission
CML (Ph+)	Chronic Myelogenous Leukaemia (Philadelphia positive)
NHL 2	Non-Hodgkin Lymphoma in 2nd remission

Conditioning for HCT

Conditioning consisted of cyclophosphamide (Cy, 60 mg/kg/day i.v. for 2 consecutive days) in all patients and was combined with TBI in children suffering from a haematological malignancy. In addition to this Cy-TBI regimen, cytarabine (1 g/m2/day for 2 consecutive days) was given to patients treated for myeloid leukaemia or myelodysplastic syndromes between 1988 and 1998 (n=1). From 1990 onward, patients treated for lymphoblastic leukaemia or non-Hodgkin lymphoma received etoposide (350 mg/m2/day for 2 consecutive days) (n=4) in addition to TBI-Cy. TBI was administered unfractionated (i.e. as one or two single fractions > 4.0 Gy), delivered at a mean dose rate of 23 cGy/min. To reduce radiation damage, lungs were compensated for their different radiation-density, and eyes were shielded during TBI from 1987 onward.

As age is an important determinant with respect to tolerability of irradiation dose in children, a TBI regimen with age-dependent total dose was applied, i.e. 0-2 years: 5.0 Gy, 2-4 years: 7.0 Gy, 4-10 years: 7.5 Gy, >10 years 8.0 Gy. The latter dose was 'increased' in 1989 to 2 single fractions of 6.0 Gy, given on 2 consecutive days, instead of the equivalent 9.0 Gy once, which had too much side effect in adults. Of the 4 patients treated for SAA, one who was transfusion-sensitised received 4 Gy TBI in addition to Cy, the other 3 received no TBI.

Growth and endocrine functions

Parameters used to evaluate growth and endocrine function were height, weight, serum levels of insulin-like growth factor 1 (IGF-1), free thyroxin (FT4), thyroid stimulating hormone (TSH), luteinising hormone (LH), follicle stimulating hormone (FSH), oestradiol, testosterone, 25-OH vitamin D, use of hormone preparations (e.g. thyroxine, oral contraception etc.) and bone mineral density (BMD). Height is measured with a stadiometer and expressed in standard deviation score (SDS) for age and sex based on Dutch references [2]. Target height was calculated from parental height with corrections for secular trend (+4.5 cm) and sex differences (13 cm) [2]. Final height (FH) SDS was compared to height SDS at the time of HCT and to target height SDS. BMD was measured with dual-emission x-ray absorption (DEXA) scan (Hologic QDR) in femoral necks and lumbar spine and results were expressed as gender specific

SD scores for young adults. Osteoporosis was defined as BMD <-2.5 SDS, Osteopenia as BMD < -1.0 SDS.

Renal function

Creatinine clearance was estimated using the formula of Cockcroft and Gault [3] with correction for body surface area. Glomerular Filtration Rate (GFR) was estimated using the Modification of Diet in Renal Disease Study 1 (MDRD1) formula, which includes age, sex, serum creatinine, serum urea and serum albumin [4]. GFR and creatinine clearance were considered normal if > 85 ml/min/1.73m^2.

Lung function

We used a standardised questionnaire to evaluate subjective pulmonary symptoms. Parameters for lung function were forced expiratory volume in one second (FEV1), functional residual capacity (FRC), vital capacity (VC), total lung capacity (TLC), residual volume (RV), and transfer factor for carbon monoxide (T_{LCO}), corrected for haemoglobin content. Results are expressed as percentage of the predicted values, derived from Quanjer et al. for VC, FEV1 [5], from Stocks et al. for TLC, FRC and RV [6], and from Stam et al. for T_{LCO} [7].

Other late effects

An ophthalmologist and a dermatologist examined patients for dermal and ocular late effects. Secondary tumours were identified from the medical records.

All laboratory evaluations were performed using standard in house, commercially available assays.

Quality of life

The sickness impact profile and the Medical Outcome Study 36-item Short Form Health Survey were used as generic questionnaires in the assessment of Quality of life (QOL). The Functional Assessment of Cancer Therapy-Bone Marrow Transplant Scale (FACT-BMT) was used as a disease-specific measure of QOL. Coping was assessed by means of the Utrecht coping list.

Results

Table 2 summarises the results of individual patients.

Growth and endocrine function

All patients had reached FH. In seven patients, pubertal growth spurt was almost completed at time of HCT (including one 12 year old boy with SAA who had received high doses of androgens for several years; his height at HCT was 186.5 cm). Of the remaining 15 patients, two had received cranial irradiation prior to TBI. Their FH's were –4.0 and –3.3 SDS. One other patient (no TBI) had received high doses of corticosteroids for several years, first for his SAA, later for severe chronic graft-versus-host-disease (GVHD). His FH was –3.5 SDS. In the remaining 12 patients, only one patient (not treated with TBI) had an increase in height SDS (+1.4 SD) between HCT and FH. In the other 11 patients (all had received TBI) median FH SDS was –2.1 (range -3.7 to 0), median difference between height SDS at HCT and FH SDS was - 1.6 (range –2.3 to –0.1), and median difference between target height SDS and FH SDS was –1.6 (range –2.6 to –0.5). None of the patients had been treated with growth hormone (GH).

Hypergonadotrophic hypogonadism was diagnosed in 10 of the 12 girls, with recovery of gonadal function in two of them. Six girls received sex hormone replacement therapy and two used oral contraception. One patient who had recovered from hypogonadism had 2 pregnancies, both resulting in spontaneous abortion. All 7 males treated with TBI and 1 of the 3 males who only received cyclophosphamide had elevated FSH levels, suggestive of severely decreased fertility. Three boys were using sex hormone replacement therapy. In one of the remaining 7 boys testosterone was decreased and LH elevated, suggestive of decreased Leydig cell function.

One patient with pre-existing hyperthyroidism before HCT underwent thyroidectomy and received thyroxine suppletion. Of the remaining patients, two had developed primary hypothyroidism after TBI and were receiving thyroxine suppletion. Free T4 and TSH were normal in all patients.

None of the patients had vitamin D deficiency. In two patients (one male), BMD measurement of the femoral neck was impossible due to hip replacement surgery after avascular hip necrosis as a result of extensive use of glucocorticosteroids for GVHD. One female patient had osteoporosis of the

lumbar spine BMD (BMD –2.6 SDS), with osteopenia of both femoral necks (-1.8 and –2.0 SDS). Another female patient had osteoporosis of her right femoral neck (BMD –2.6 SDS), with osteopenia of the other hip and lumbar spine (BMD –2.4 and –1.9 SDS respectively). Of the remaining patients, 9/18 had osteopenia of one or both hips, and 6/20 had osteopenia of the lumbar spine.

Renal function

Median estimated creatinine clearance was 100 ml/min/1.73m^2 (range 83-124). Median estimated GFR using the MDRD1 formula was 99 ml/min/1.73m^2 (range 76-131). One patient had a decreased estimated creatinine clearance (83 ml/min/1.73m^2) but normal estimated GFR (88 ml/min/1.73m^2). Another patient had a decreased estimated GFR (76 ml/min/1.73m^2) but normal estimated creatinine clearance (89 ml/min/1.73m^2). In both patients actual creatinine clearance (measured using 24-hr urine samples) was normal (101 and 141 ml/min/1.73m^2 respectively).

Lung function

Apart from the patient with severe pulmonary fibrosis after a re-transplantation, 3 patients reported mild shortness of breath during exercise. The remaining 18 patients were free of pulmonary symptoms. Lung function tests were missing in one female patient due to a technical error. One patient had severe pulmonary fibrosis due to radiation damage and pulmonary GVHD, which resulted in oxygen dependency and a VC of only 20% of predicted. This patient was excluded from the analyses. Median VC was 80% (54-115), median TLC was 83% (56-125), median RV was 70% (42-222) median RV/TLC was 92% (56-158), median FEV1/VC was 106% (88-124) and median T_{LCO} was 60% (40-104). TLC, VC and T_{LCO} were significantly decreased in our population (one-sample Student t-test, $p<0.001$), whereas FEV1/VC was significantly increased ($p=0.015$). All patients with pulmonary symptoms had abnormal pulmonary function tests.

Other late effects

Of the 19 TBI treated patients, 12 received TBI before 1987, which means that they did not have their eyes shielded to reduce the radiation dose to the

lenses. Two of these patients had a history of lens extraction for severe cataract after HCT. In the other 10 patients, mild cataract was found. The one patient who received a relatively low dose of 4 Gy TBI also had mild cataract. She had also received high doses of corticosteroids during the initial treatment of her SAA. Of the 7 patients receiving TBI after 1987 (i.e. with eye shielding), only one had very mild cataract. Of the 3 patients who did not receive TBI, one patient, treated with corticosteroids for GVHD for a long time, had mild cataract. Two patients with chronic GvHD had keratoconjunctivitis sicca.

Physical examination by a dermatologist revealed no dysplastic naevi in any of the patients. One patient was treated for basal cell carcinoma in the past, and in one patient a basal cell carcinoma was diagnosed and excised. Four patients had sclerotic skin lesions as a result of chronic GVHD.

Three patients (all treated with TBI) had developed a secondary tumour (one thyroid carcinoma and 2 basal cell carcinoma, as described above).

Quality of life

Results of the QOL measurements are published elsewhere [8]. Of the generic QOL measures, most results fell within the normal range of functioning, although some illness-related impairment was reported on subscales for general and work-related functioning. Compared to a reference sample of patients who had received BMT as adults, patients involved in this study scored significantly higher on the ‘emotional well-being’ subscale of the FACT-BMT, indicating significantly better emotional functioning. The age at BMT and total body irradiation (TBI) were not related to patients’ QOL.

Table 2. Late effects in individual patients

Pat. No.	Sex (m/f)	Indication HCT	TBI dose (Gy)	FH (SDS)	FH-TH (SDS)	Gonadal dysfunction	Hypo-thyroidism	BMD	Renal function	Lung function	Cataract	GVHD	2nd tumour
1	m	SAA	0	***	*			*	*		*	**	
2	m	SAA	0		*			*		*	*	**	
3	m	SAA	0			*							skin
4	f	SAA	4.0		*	*		*		*	*		
5	m	ALL 1	6.0 x 2			*		*					
6	F	ALL 1	6.0 x 2	*		*		**					
7	m	ALL 2	7.5	***	***	*		*			*		
8	m	ALL 2	7.5	***	***	*	*	*		*	*	*	
9	f	ALL 2	6.0 x 2			*							
10	f	ALL 2	8.0	*		*		*		*	*	**	thyroid
11	f	JMML	5.0	**	**						*		
12	f	AML	7.5	**	**		*			*	**		
13	m	AML	7.5	***	**	*				*	*		
14	f	AML	8.0	**	*	(*)		*		*	*		
15	f	AML	8.0	*	*	*				*	*		
16	f	AML	8.0			(*)		*		*	*		
17	f	AML	8.0	**	*	*				*			
18	f	AML	8.0			*		*	*	missing	**		skin
19	m	AML	8.0	*	**	*		*		**	*		
20	m	MDS	7.5	***	**	*		*		*	*		
21	f	CML Ph^+	7.5	**	*	*		**		*	*	*	
22	m	NHL	6.0 x 2		*	*		*		*	*		
				* <-1 sd ** < -2 sd *** <-3 sd		* hypogonadism (*) recovered		* osteopenia ** osteoporosis	* < 85 ml/min/ 1.73m^2	* < 75% ** <50%	* mild ** severe	* limited ** extended	

Discussion

Impaired growth is an important late complication of HCT, with TBI as the most important etiological factor. TBI may have a direct effect on growth (by inducing growth plate damage) as well as an indirect effect (by causing GH deficiency, hypogonadism or hypothyroidism). The indirect effects can be overcome by hormone replacement therapy. Other factors that may contribute to impaired growth are chronic GVHD, its treatment with glucocorticosteroids, and cranial irradiation prior to HCT, resulting in GH deficiency.

In our evaluation, median adult height after TBI was 1.6 SDS (±11 cm) lower compared to both target height and height at HCT. In patients who also had received cranial irradiation, adult height was even more compromised. These results are comparable to final heights reported in the literature [9-12].

Except for the two girls who were the youngest at the time of HCT, all women who had received TBI developed hypergonadotrophic hypogonadism after TBI. In two of these women gonadal function recovered, and one of them became pregnant twice. These results are in line with the literature, reporting a positive correlation between age at HCT and incidence of ovarian failure [13-15]. As could be expected from earlier reports [16], TBI resulted in decreased fertility in men. In addition, with increasing age, signs of Leydig cell failure became more evident, with elevated serum levels of LH in 4 of 7 men.

Hypothyroidism after HCT is related to radiation damage to the thyroid gland. The incidence of hypothyroidism after TBI in this study was 2/18 (11%) which is relatively low compared to incidences reported in the literature (45% after sf-TBI and 15% after fractionated TBI) [16]. An explanation for this lower incidence could be the relatively low total TBI dose in our study (most patients received 7.5-8.0 Gy, compared to 10 Gy in most studies with sf-TBI).

Osteopenia was present in 64% of the patients, two of whom also had osteoporosis. As most patients had a relatively low adult height, BMD SDS is probably slightly higher if corrected for height. Unfortunately, these data were not available. The percentage of patients with reduced BMD in our study is comparable to that reported in adults by Kauppila et al. (68%) [17]. The most important factor contributing to the decreased BMD is the use of glucocorticosteroids in patients with chronic GVHD. In addition, hypogonadism (especially in girls with ovarian failure), non-compliance in patients with sex

hormone replacement therapy, and TBI also contribute to the decreased BMD [16]. The small population size prevented further analyses of these influencing factors.

Renal function was normal in all 22 patients. Decreased renal function is related to the use of nephrotoxic agents (e.g. cisplatin, cyclosporine, amphoterecin B) and total TBI dose. The reported incidence of renal dysfunction in adults receiving fractionated TBI at total doses of less than 12 Gy is approximately 5% [18;19]. Recently, results of a prospective study into chronic renal failure after HCT were reported by our transplant centre [20]. In that study, TBI was not a risk factor for either acute or chronic renal failure in the first 2 years after HCT. Frisk et al., however, report repeated measurements of GFR and describe a gradual decrease in GFR with time after HCT [21]. They report renal insufficiency (GFR < 70 ml/min/1.73m^2) in as much as 27% of 26 patients after paediatric HCT and 7.5 Gy single-fraction TBI (median follow-up of 10 years), compared to 0% of the 14 patients receiving radiation-free conditioning for HCT. We do not have an explanation for the low incidence of renal insufficiency after TBI in our patients, but differences in the use of nephrotoxic agents during treatment of initial disease and during the transplantation period may play a role.

There was a decrease in lung function parameters compatible with restrictive pulmonary disease in 66% of our population (i.e. decreased TLC and RV, increased FEV1/VC). In addition, diffusion capacity, measured by T_{LCO} was also significantly decreased (<80% of predicted) in 10/20 (50%) patients. None of the patients had obstructive lung disease. The majority of the patients (82%) were free of symptoms. Restrictive lung disease and decreased diffusion capacity is associated with TBI, and in longitudinal studies lung function stabilises after initial decrease [11;22-25]. In contrast to the high incidence of restrictive lung disease in our population, Frisk et al. reported an incidence of only 21% 10 years after 7.5 Gy sf-TBI [11] , and Cerveri et al. reported 26% two years after 6x2.0 Gy fractionated TBI [24]. These differences can be explained by the absence of GVHD in the first study (autologous transplants only) and the shorter follow-up and possibly the use of fractionated TBI in the latter study.

The incidence of cataract, a well known late effect of ocular irradiation, depends on total dose, dose rate and fractionation. Our results are comparable to those reported in the literature [26;27]. The study of Kempen-Harteveld et al. [27]

was a national study on paediatric HCT recipients and included all patients receiving TBI in our centre until 2001. Before eye shielding was applied, the incidence of cataract after sf-TBI was >90%, whereas with eye shielding it was approximately 30%.

Three patients had developed a secondary malignancy. Based on both animal data and the experience with radiation induced tumours in humans, more secondary tumours can be expected in the future. [28;29]

Our cross-sectional evaluation revealed a substantial number late of effects in a wide variety of organ systems, and all patients in our study suffered from more than one such late complication. Therefore life-long follow-up of all patients treated with HCT is indicated. Shielding of radiosensitive organs may reduce the incidence of late effects (e.g. eye-shielding to prevent cataract), but is not feasible for many organs (e.g. gonads) in children treated for malignancies. Growth hormone therapy is a promising option for the treatment of radiation induced growth impairment (own unpublished data), but multi-centre studies are needed to determine its benefits and risks in this category of patients.

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FINAL HEIGHT OF PATIENTS WHO UNDERWENT BONE MARROW TRANSPLANTATION FOR HAEMATOLOGICAL DISORDERS DURING CHILDHOOD: A STUDY BY THE WORKING PARTY FOR LATE EFFECTS-EBMT

Blood 1999;93:4109-4115

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Abstract

Few data are available on the long-term effect of bone marrow transplantation (BMT) on growth. This study examines those factors that play a role in the final height outcome of patients who underwent BMT during childhood. Data on 181 of 230 patients with aplastic anaemia, leukaemia, and lymphomas who had BMT before puberty (mean age 9.8 ± 2.6 years) and who had reached their final height were analysed. An overall decrease in final height standard deviation score (SDS) value was found compared with the height at BMT ($P < 10^7$) and with the genetic height ($P < 10^7$). Girls did better than boys, and the younger in age the person was at time of BMT, the greater the loss in height. Previous cranial irradiation + single-dose total body irradiation (TBI) caused the greatest negative effect on final height achievement ($P < 10^4$). Fractionation of TBI reduces this effect significantly and conditioning with busulphan and cyclophosphamide seems to eliminate it. The type of transplantation, graft-versus-host disease, growth hormone, or steroid treatment did not influence final height. Irradiation, male gender and young age at BMT were found to be major factors for long-term height loss. Nevertheless, the majority of patients (140/181) have reached adult height within the normal range of the general population.

Introduction

The success of bone marrow transplantation (BMT) in treating malignant and non-malignant haematological disorders and the improvement of the sophisticated techniques involved in this procedure have extended the indications for transplantation and have increased the number of patients who survive BMT [1;2]. Nevertheless, one of the many negative effects that these successfully treated children have to face is the endocrine dysfunction associated with the chemotherapy, radiotherapy, and immunosuppressive treatment they receive before and after marrow transfusion, which could eventually induce growth delay. To our knowledge, only two single-centre studies have dealt with the final height achievement of patients who had BMT during childhood [3;4]. Because the BMT procedure is relatively recent, making it difficult to include large numbers of patients who have reached their final adult height, the statistical power of these two studies was frail. The European BMT Working Party for Late-Effects conducted this multi-centre study with the aim of evaluating the final height achieved by children who underwent BMT for haematological disorders and identifying those factors that influence the long-term growth in these patients.

Patients and methods

Study design

The study is based on a retrospective survey using a two-step-questionnaire approach, involving centres that are part of the European-BMT group.

A first questionnaire was sent to 284 BMT centres asking for the number of patients with severe aplastic anaemia (SAA), leukaemia, and lymphomas who underwent BMT before onset of puberty (breast stage 1 in girls and testicular volume less than 4 mL in boys) and who had reached their final adult height. Final height was defined either on a documented closure of the hand, wrist, or iliac crest epiphyses or growth velocity less than 1 cm/yr [5].

One hundred of the 284 centres (35%) completed and sent back the first questionnaire form. Sixty-two centres (22%) confirmed that they did not have cases that met the inclusion criteria. A second questionnaire was sent to the

remaining 38 centres that claimed to have patients eligible for the study. Twenty-two of the 38 centres answered the second questionnaire, providing data on a total of 230 patients.

The questionnaire included queries regarding the primary haematological disorder, irradiation therapy used during first-line treatment (between diagnosis and pre-BMT conditioning treatment), BMT-related data (age and type of BMT, conditioning regimen, grading of acute and chronic graft-versus-host disease [GVHD], and type and duration of immunosuppression therapy), and endocrine-related data that included parental height, patient's height and weight at BMT, final adult height achieved, age at latest measurement, growth hormone treatment, and sex hormone replacement therapy performed.

Patients' characteristics

Of the 230 forms received, 49 patients were excluded from the study either because the onset of puberty was before BMT or because of insufficient key data necessary for a correct and comprehensive statistical analysis. BMT was performed between October 1973 and October 1993. The characteristics of the 181 patients who met the inclusion criteria are summarised in table 1.

Table 1. Patients' characteristics at diagnosis and at transplantation

Patients studied	181 (112 M; 69 F)
Mean age at diagnosis (yrs)	8.1 ± 3.3 (range 0.9-14.4)
Diagnosis and disease status at BMT	
- ALL	73 (15-1st CR: 45-2nd CR: 11-≥2nd CR; 2 relapse)
- AML 1st CR	46
- CML chronic phase	10
- Myelodysplastic syndrome	2
- NHL	2
- SAA	48
Mean age at BMT (yrs)	9.8 ± 2.6 (range, 1.5-14.9)
Type of BMT	
- Allogeneic	153 (149 identical, 2 unrelated, 2 mismatched related)
- Syngeneic	3
- Autologous	25
GVHD	
- Acute-grade 3-4	16
- Chronic-limited	37
- Chronic-extended	18
Mean follow-up period (yrs)	9.2 ± 3.1 (range 2.9-19.7)

The type of irradiation applied to the patients in relation to the primary disorder is shown in table 2. Fifty patients received cranial radiation therapy (CRT) as prophylaxis or treatment of central nervous system involvement (<18 Gy in 4 patients, 18 Gy in 29 patients, 24 Gy in 16 patients, and 36 Gy in 1 patient). Irradiation during conditioning regimen included single-dose total body irradiation (sTBI) in 52 patients at a median dose of 8 Gy (range, 3 to 10 Gy; 3 SAA patients received 3 to 4 Gy, whereas the remaining patients received 7 to 10 Gy); fractionated TBI (fTBI) in 73 patients (6 to 13.2 Gy) administered in 2 to 8 fractions; thoraco-abdominal irradiation (TAI) in 17 patients (5 to 11 Gy); and total lymphoid irradiation (TLI) in 2 patients (7.5 Gy). Seventeen children with acute lymphoblastic leukaemia (ALL) received a booster dose of 4 to 10 Gy to the testicles.

Table 2. Irradiation applied to the patients in relation to the primary disease.

			First line irradiation therapy				**Irradiation during conditioning**				
Diagnosis	No. of patients	Non-Irradiated	CRT	Cranio-spinal	Testes	Ocular	sTBI	fTBI	TAI	TLI	Boost
ALL	73	0	42	4	10	3	25	47	1	-	17
AML	46	7	3	-	-	-	20	17	1	-	-
CML	10	2	1	-	-	-	1	7	-	-	-
MDS	2	0	-	-	-	-	2	-	-	-	-
NHL	2	0	-	-	-	-	1	1	-	-	-
SAA	48	27	-	-	-	-	3	1	15	2	-
TOTAL	181	36	46	4	10	3	52	73	17	2	17

Patients subjected to irradiation as part of the pre-BMT conditioning regimen also received cyclophosphamide (Cy) alone or in combination with other cytotoxic drugs (cytarabine, etoposide, and vincristine). Of the 36 patients who did not receive irradiation, 10 children (7 acute myeloid leukaemia [AML], 2 chronic myeloid leukaemia [CML], and 1 SAA) were conditioned with busulphan and cyclophosphamide only (Bu/Cy). Steroid therapy for acute and/or chronic GVHD was administered in 87 patients for a median period of 4 months (range, 0.5 to 168 months); 62 of them stopped treatment within 12

months, whereas 14 patients had treatment for periods longer than 24 months. Cyclosporin-A was administered in 90 patients for a median period of 6.5 months (range 2 to 84 months); 67 of them stopped treatment within 12 months, whereas 11 patients had treatment for periods longer than 24 months.
Sex hormone replacement therapy was administered to 55 patients (24 male and 31 female), starting at 14.0 ± 3.3 years of age (range, 12 to 18 years of age) in males and at 14.4 ± 1.8 years of age (range 11 to 18.8 years of age) in females. Growth hormone (GH) treatment was administered in 28 patients for a median period of 3.5 years (range 0.3 to 7 years), starting at 13.2 ± 2.1 years of age (range 9.8 to 17.9 years of age), 3.8 ± 2.0 years from BMT (range, 0.9 to 8.3 years).

Statistical analyses

Height measurements of each patient both at the time of BMT and final height were expressed as the standard deviation score (SDS) from the mean of the normal population [5]. The genetic height of each patient was calculated as the mean of the mother's and the father's height-SDS (genetic height = [mother's SDS + father's SDS]/2).
The difference between the height-SDS value at BMT and that of the final height was calculated for each patient and was regarded as the delta-SDS value, expressing the gain (zero or positive values) or the loss of height (negative values) after transplantation in terms of SDS.
Statistical analyses for the comparisons of delta-SDS values were performed according to the type and age at BMT, gender, pre-transplant conditioning regimens, complications, and therapies applied. The relationships between dependent and explanatory covariates were investigated by using the analysis of variance and the χ^2 statistics for continuous and categorical covariates, respectively.
The association between delta-SDS, as dependent variable, with the type of BMT, age at transplant, gender, radiotherapy, chronic GVHD severity, and GH treatment were also investigated using the multiple logistic regression analysis [6]. This multivariable technique permits identification of covariates that are associated with the probability of the studied outcome and expresses each covariate association, adjusted for the effect of the other covariates included in the regression model, in terms of relative risk point estimates (RR) and its confidence intervals.

To this aim, the dependent variable delta-SDS was dichotomized to distinguish between subjects who had normal growth after transplant (i.e., delta-SDS value ≥0) and those who had growth failure (delta-SDS <0). Patients were also divided into three groups to identify subjects who received CRT (with or without TBI), subjects who received radiation therapy that did not include CRT, and patients who have never received any irradiation therapy (reference group, RR = 1). The age at transplant (continuous covariate) was categorised into three levels according to the 33rd (1.5 to 8.8 years) and the 66th percentile values of its frequency distribution (8.8 to 11 years). Patients with age greater than 11 years at transplantation were used as a reference (i.e., RR = 1). The statistical analyses were performed using the SPSS statistical software, version 8.0 (SPSS Inc, Chicago, IL) [7].

Results

Final height achievement was documented by closure of hand, wrist, or iliac crest epiphyses in 9 patients who were 18.5 ± 2.4 years of age at latest evaluation and by growth velocity less than 1 cm/yr in the remaining patients, who were 19.1 ± 2.8 years of age. Final height-SDS values of 140 of 181 were within normality for the general healthy population (between -2.0 and +2.0 SDS). Three patients achieved height values greater than +2.0 SDS, whereas the remaining 38 are to be considered as short stature (below -2.0 SDS).

Considering the entire cohort of patients (Fig 1), the height-SDS value at BMT (-0.15 ± 1.16) was significantly higher (paired Student's t-test; $P < 10^7$) than the final height-SDS value (-1.09 ± 1.45), resulting in a mean decrease of 0.94 ± 1.30 SDS from transplant to adulthood.

Whereas the height-SDS value at BMT was comparable to that of the genetic height (-0.22 ± 1.02 SDS), the final height-SDS value was significantly lower ($P < 10^7$). The mean delta-SDS value of the whole cohort was -0.94 ± 1.30 (range -6.9 to +2.6). The 112 boys did worse than the 69 girls, having a mean delta-SDS value of -1.17 ± 1.34 compared with -0.56 ± 1.13, respectively (t-test; P < 0.002). Girls were younger at BMT compared with boys (8.9 ± 2.7 and 10.3 ± 2.4 years, respectively; P<0.0005). Age was found as an additional factor that influences growth, because the younger the age at BMT the higher the delta-SDS value (linear regression analysis; regression coefficient = 0.218; $P < 10^7$) and the lower the final height-SDS (regression coefficient = 1.104; P = 0.01).

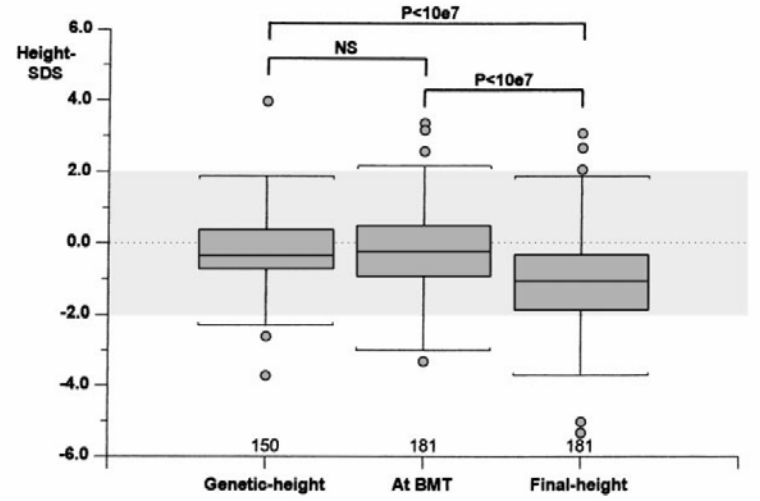

Figure 1. Correlation between the genetic height, height-SDS at BMT, and final height-SDS. Numerals indicate the number of cases studied in each group. The dotted area indicates the height-SDS distribution for the normal general population. Box plot: The lower line of the box indicates the 25th percentile, the upper line indicates the 75th percentile, and the horizontal lines above and below the boxes represent the 3rd and the 97th percentile, respectively. Statistical analyses: paired Student's t-test.

The type of BMT was found to have no effect on the delta-SDS value (analysis of variance, one-way ANOVA); in fact, the 28 patients who underwent autologous-syngeneic BMT (for statistical purposes, the 3 children who received a transplantation from a monozygotic twin were considered as part of the autologous group) had a delta-SDS value of -0.95 ± 1.25 compared with that of -0.94 ± 1.31 in the 153 cases who had allogeneic BMT.

Seven different groups were identified according to the type of irradiation and chemotherapy applied (table 3); the age at BMT was similarly distributed in these groups (analysis of variance).

As shown in Fig 2, the most severe growth failure was found in patients who received CRT+sTBI (mean delta-SDS value, -2.07 ± 0.91) followed, respectively, in decreasing degree of severity by sTBI (-1.37 ± 1.06), CRT+fTBI (-1.11 ± 1.61), fTBI (-0.88 ± 1.25), and TAI/TLI (-0.71 ± 0.72). The non-irradiated group had virtually no growth deficit after BMT (-0.07 ± 1.08).

Table 3. Characteristics of patients divided according to the irradiation protocol applied.

Type of irradiation	No. of Patients	Age at BMT (yr) (mean ± SD)	Delta-SDS (mean ± SD)	Final height-SDS (mean ± SD)
CRT	3	11.2 ± 0.4	-0.93 ± 1.10	-1.20 ± 2.16
CRT+sTBI	13	8.4 ± 2.2	-2.07 ± 0.91	-2.42 ± 1.22
CRT+fTBI	34	10.2 ± 2.3	-1.11 ± 1.61	-1.69 ± 1.68
sTBI	39	9.5 ± 2.5	-1.37 ± 1.06	-1.15 ± 1.06
fTBI	39	10.0 ± 2.9	-0.88 ± 1.25	-0.98 ± 1.21
TAI/TLI	17	8.7 ± 2.2	-0.71 ± 0.72	-0.85 ± 0.93
No irradiation	36	10.3 ± 2.9	-0.07 ± 1.08	-0.21 ± 1.54
- Cy only (SAA)	26	9.8 ± 2.8	-0.12 ± 1.08	-0.15 ± 1.68
- Bu/Cy	10	11.7 ± 2.7	+0.05 ± 1.13	-0.36 ± 1.16

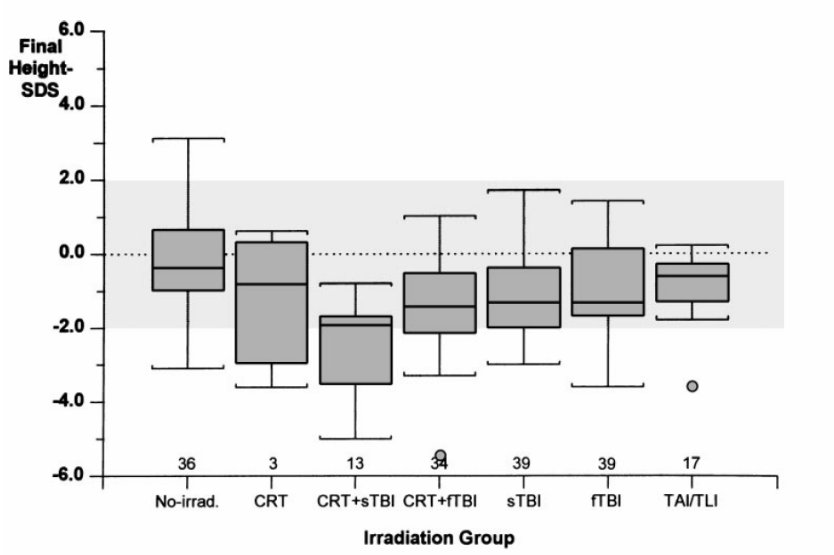

Figure 2. Delta-SDS (final height SDS minus SDS at BMT) in the different irradiation groups. Numerals indicate the number of cases studied in each group. (Box plot) The lower line of the box indicates the 25th percentile, the upper line indicates the 75th percentile, and the horizontal lines above and below the boxes represent the 3rd and the 97th percentile, respectively.

A similar pattern was found when final height-SDS values were considered (Fig 3). The delta-SDS value of the 10 non-irradiated patients conditioned with Bu/Cy (+0.05 ± 1.13) was not statistically different from the 26 non-irradiated SAA patients (-0.12 ± 1.08).

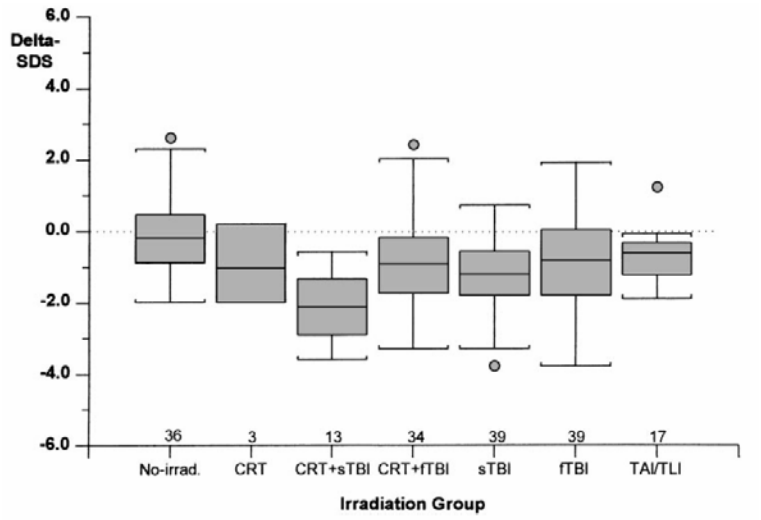

Figure 3. Final height-SDS achievement in the different irradiation groups. Numerals indicate the number of cases studied in each group. The dotted area indicates the height-SDS distribution for the normal general population. Box plot: The lower line of the box indicates the 25th percentile, the upper line indicates the 75th percentile, and the horizontal lines above and below the boxes represent the 3rd and the 97th percentile, respectively.

Comparing the delta-SDS value among the patients divided into groups according to diagnosis, no significant difference was found between the ALL (73 cases; -1.26 ± 1.35), AML (46 cases; -0.96 ± 1.06), and CML groups (10 cases; -0.74 ± 1.98). The delta-SDS found in the SAA group (48 cases; -0.43 ± 1.09) was significantly better than that of the ALL and AML groups. Dividing the SAA group into non-irradiated (27 cases; -0.12 ± 1.06 delta-SDS) and irradiated (21 cases; -0.82 ± 1.01), a significant statistical difference was found (Wilcoxon rank-sum test; $P < 0.03$).

The severity of chronic GVHD (126 cases with no GVHD: -0.84 ± 1.20 delta-SDS; 37 cases with limited GVHD: -1.08 ± 1.30; and 18 cases with extended

GVHD: -1.34 ± 1.85) did not significantly influence the delta-SDS mean value (analysis of variance: P = 0.22), even though an increasing severity of the chronic GVHD showed a tendency toward worsening of delta-SDS.

On a long-term basis, cortisone treatment (87 treated patients [-1.03 ± 1.48 delta-SDS] v 84 not treated [-0.79 ± 1.07]) and cyclosporin-A treatment for GVHD (91 treated [-0.84 ± 1.22] v 80 not treated [-0.99 ± 1.38]) were found to have no effect on delta-SDS values.

The 55 patients who received sex hormone replacement therapy reached a similar final height-SDS (-1.05 ± 1.53) compared with the group of patients who started and completed pubertal development spontaneously (-1.05 ± 1.35).

The mean delta-SDS found in the 28 patients treated with exogenous GH (-1.14 ± 1.24) was not statistically different from that of the remaining 153 patients (-0.90 ± 1.31) not treated with exogenous GH. Because irradiation was the major factor in altering the delta-SDS value in our cohort, this analysis was performed within the same conditioning group. Twelve of 34 patients who received CRT+fTBI and who were treated with GH (delta-SDS -0.75 ± 1.32) were compared with the remaining 22 patients who did not receive GH treatment (delta-SDS -1.31 ± 1.75); 8 of 13 patients who received CRT+sTBI and who were treated with GH (delta-SDS -1.83 ± 0.71) were compared with the remaining 5 patients who did not receive GH treatment (delta-SDS -2.46 ± 1.13); and 7 of 39 patients who received sTBI and who were treated with GH (delta-SDS -1.24 ± 1.38) were compared with the remaining 32 patients who did not receive GH treatment (delta-SDS -1.40 ± 1.0). None of the comparisons was found to be statistically significant, but there seems to be a trend towards better growth in the GH-treated group. We also failed to show differences within the same gender, both between GH-treated (20; -1.21 ± 1.34 delta-SDS) and untreated boys (92; -1.77 ± 1.35) and between GH-treated (8; -0.99 ± 1.01) and untreated girls (61; -0.50 ± 1.14 delta-SDS).

Multiple-logistic regression was used to model the relationship between the dependent variable delta-SDS and the explanatory covariates (gender, age at transplant, type of BMT, irradiation applied, chronic GVHD severity, and GH therapy). Stepwise multiple logistic regression identified irradiation, age at transplant, and gender as statistically relevant explanatory covariates that significantly contributed to the model that was fitted to the data (table 4).

The role of each covariate in determining a relevant growth deficiency (delta-SDS <0) while accounting for the effect of the other covariates included in the

logistic regression model and the estimated effect size is reported as relative risk point estimates with their 95% confidence intervals (table 4).

Table 4. Stepwise regression logistic analysis identifying the role of each covariate in determining a relevant growth deficiency (delta-SDS less than 0) while accounting for the effect of the other covariates. The estimated effect magnitude is reported as relative risk point estimates (RR) with their 95% confidence intervals (CI).

Covariate		Relative Risk for growth failure	95% CI	*P* value
Irradiation				
No irradiation	(36)	1.0	Reference level	0.0002
CRT ± TBI*	(50)	6.96	2.11-22.95	
TBI/TAI/TLI§	(95)	6.89	2.55-18.57	
Gender				
Female	(69)	1.0	Reference level	0.0069
Male	(112)	4.52	1.75-11.66	
Age (years)				
>11	(60)	1.0	Reference level	0.01
8.8-11	(61)	2.61	1.73-16.23	
<8.8	(60)	5.29	0.95-7.19	

* Patients who received CRT during first line therapy.

§ Patients who did not receive CRT but who had either single or fractionated TBI, TAI or TLI during pre-BMT conditioning.

Discussion

The normal growth process during childhood reflects the child's general well-being, and it is regulated by and depends on the interaction between genetic, nutritional, metabolic, and hormonal factors. Nevertheless, growth is not always linear, especially in children who have periods of chronic illnesses and/or undergo toxic treatment procedures. The end result of growth is the final adult height, which is used in this study as the long-term marker for treatment-related toxicity in patients who underwent BMT during childhood.

Growth impairment in the short term has been repeatedly reported after BMT [8-12], but data on final height achievement are scarce, and the only two published reports dealt with a limited number of patients [3;4]. This is the first multi-centre

study on final height with a large number of patients that takes into consideration the various potential risk factors that might affect growth after transplantation performed during childhood. Solid tumours and haematological disorders in which short stature is a trait of the disease itself (Fanconi's anaemia, Thalassemia, inborn errors, etc) were excluded.

Our data showed a similarity between the genetic height and the height at BMT on one hand and a decreased value of final height-SDS compared both with the genetic height and the patient's height-SDS at BMT on the other, suggesting that the growth impairment in transplanted patients occurred mostly during the period after transplantation (Fig 1).

The outcome of final height in this study did not change significantly between the different types of haematological malignancies (ALL, AML, and CML), suggesting that, in this cohort of patients, the primary disease itself does not affect growth. This study confirms that irradiation is the major contributor for long-term growth impairment (final height achievement). Patients who were not irradiated had virtually no decrease in final height-SDS compared both with the height at BMT and the genetic height, underlining what was reported in smaller series of patients [3;4]. Among the different irradiation settings, leukaemia patients who received CRT during first-line treatment and sTBI during pre-BMT conditioning had the most severe long-term impairment of growth (Figs 2 and 3). Moreover, patients with an identical primary disorder (SAA) who were treated with two different conditioning regimens (Cy+irradiation v Cy only) presented two completely different patterns of growth, with the most favourable being the non-irradiated group.

Because the Bu/Cy conditioning regimen has been more recently introduced to reduce the detrimental effects of irradiation [13], the number of the Bu/Cy patients in this study is too small to draw unequivocal conclusions regarding the effect of this regimen on the long-term growth. Nevertheless, and notwithstanding these limitations, final height achieved by these patients was similar to their predicted final height, suggesting that, despite the known radio-mimetic effect of busulphan [14], Bu/Cy pre-BMT conditioning regimen has less interference on the growth process than does irradiation. Published data available on the effect of Bu/Cy regimen on growth are discordant, and report experience on the short-term growth, but not on final height achievement. Whereas Wingard et al. [15] reported on the similarity between the effect of Bu/Cy and TBI, 2 years after transplant, 8 of 24 patients in that study who were

conditioned with Bu/Cy also received CRT during first line treatment. Other studies [16-18] found no harmful effect of Bu/Cy on growth. However, these three studies were based on a short-term follow-up (3 to 6 years), whereas the present study reports the final height outcome of Bu/Cy conditioning, albeit on a limited number of patients. Unfortunately, although Bu/Cy conditioning should be encouraged, at least in paediatric patients, the attempt to substitute a TBI-based conditioning regimen with Bu/Cy was not found to be advantageous when applied to patients with ALL [19].

The type of BMT (autologous or allogeneic) did not influence final height achievement. In this context, because chronic GVHD is a relatively common complication in patients who receive allogeneic BMT [20] and is not encountered after autologous BMT, the severity of chronic GVHD and its treatment (steroids and cyclosporin-A) were also found to have no significant effect on growth, even though a tendency toward worsening delta-SDS with increasing severity of chronic GVHD was documented. Although steroids and severe chronic systemic illness (i.e., chronic GVHD) are known to induce growth impairment in children, our study, however, despite being limited by a relatively small sample size, suggests that children surviving after transplantation have an adequate, although partial, capacity to catch-up with growth in the long term.

Being younger at BMT, the female group was theoretically supposed to experience greater growth impairment than males. Nevertheless, the loss in height-SDS was more profound in boys than in girls, although the two groups were comparable for differences in genetic heights and height at BMT, sex hormone replacement therapy, and age of commencement of sex hormone treatment. This phenomenon therefore remains open for further specific studies.

The loss in growth velocity in patients after BMT seems to be the result of a complex interaction of different factors related to the effect of irradiation and chemotherapy, such as lesions of bone, cartilage, and the epiphyseal growth plate; gonadal damage; delayed or precocious puberty; and hypothyroidism. Growth delay has also been attributed to GH deficiency [8-12;18;21]. Data on GH secretion were not included in the questionnaire, and GH therapy was prescribed by some of the BMT centres. Despite the relatively small number of patients who received GH treatment in our cohort, the effect on the final height outcome was less enthusiastic than that reported by others. Thomas et al [22] showed that growth impairment after BMT in a homogeneous group of 49

children with leukaemia who received CRT and TBI resulted prevalently from severe spinal growth suppression (reduced spinal height) that was unresponsive to GH treatment; also, there was an inappropriate response with absent catch-up growth in their legs. Even in children surviving brain tumours (a group with florid radiation-induced GH deficiency), GH treatment increased the short-term growth velocity but did not significantly improve the final height [23]. Furthermore, in our cohort, a reduced final height was also observed in patients with SAA irradiated with TAI/TLI only, i.e., with irradiation fields not involving the skull and its neuro-endocrine structures. This observation is further emphasised by the finding that patients who received CRT with or without TBI (high cumulative irradiation dose to the hypothalamic-pituitary region) had an equal relative risk for developing growth failure, as those patients who had irradiation that did not include CRT (stepwise multiple logistic regression analysis). GH deficiency, therefore, does not seem to play a major role in growth impairment after BMT. Because patients are already at high risk for secondary tumours after BMT [24;25], and although available data on the safety of GH-treatment in patients with a history of malignancies are reassuring [26], we recommend caution in selecting patients as candidates for GH treatment after BMT, especially because a positive long-term effect of GH treatment on growth is not yet ascertained in such patients. Furthermore, because we found that, in the long term, 140 of 181 patients who attained their final height reached normal heights (within ±2 SDS for the general population), we also suggest that growth should be clinically followed-up once every 6 months and that only a few selected cases of severe and persistent growth deficiency, observed after the interruption of the post-transplant medication, be considered for GH treatment.

This study gathered data on patients who were transplanted during a period when CRT was frequently used as prophylaxis treatment in the majority of ALL patients and single-dose administration of irradiation was widely used. At present, CRT is used in a small and selected group of children, and irradiation schedules encourage fractionated TBI. Because irradiation was found to have a significant role in long-term growth impairment, especially in patients who received cranial irradiation before transplant and received sTBI during pre-BMT conditioning, we expect an improvement in the height prognosis in children transplanted during the 1990's.

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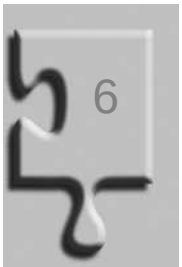

PUBERTAL DEVELOPMENT AND GROWTH AFTER TOTAL-BODY IRRADIATION AND BONE MARROW TRANSPLANTATION FOR HAEMATOLOGICAL MALIGNANCIES

European Journal of Pediatrics 2000;159:31-37

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Abstract

Pubertal development after total-body irradiation (TBI) was investigated in 40 children (21 boys) treated with allogeneic bone marrow transplantation (BMT) for haematological malignancies at a mean age of 11.3 years. The mean age at the last visit was 19.0 years. Twenty-five patients (15 boys) were prepubertal at the time of BMT. Data on secondary sexual characteristics, the pituitary-gonadal axis and longitudinal growth were retrospectively collected from the medical records. In boys not receiving additional testicular irradiation (n = 19), penile growth and pubic hair development was normal and all had serum testosterone levels within the adult range. The majority of them, however, had incidental elevations of LH, suggesting minor Leydig cell damage. Testicular volume at last measurement was small (mean: 10.5 ml) and serum FSH levels were elevated in all boys, with normalisation in only one, suggesting severe impairment of reproductive gonadal function. Of the ten girls who received BMT before puberty, six had a spontaneous onset of puberty and menarche; the four other girls needed hormonal substitution therapy. Recovery of gonadal function after cessation of substitution was seen in one girl, who became pregnant but had a spontaneous abortion. Decrease in height SDS was seen in the majority of patients and was positively correlated with male gender and lower age at the time of BMT.

Conclusion: Careful monitoring of both gonadal function and growth after bone marrow transplantation and total body irradiation is warranted in order to detect disturbances early and ensure normal pubertal development in children treated for haematological malignancies.

Introduction

Over the last two decades bone marrow transplantation (BMT) has become an important treatment modality for haematological malignancies, and a combination of total-body irradiation (TBI) and high-dose chemotherapy is frequently used in preparative regimens for BMT. The main aims of these preparative regimens are: (a) myeloablation in order to enable grafting of donor marrow and (b) the eradication of malignant cells that might have survived previous treatment. Unfortunately, TBI and high-dose chemotherapy have important negative effects on several organs including those of the endocrine system, which may lead to disturbances in both growth and pubertal development after BMT for childhood malignancies [1-3]. As the number of patients with long-term disease-free survival increases, more patients who received BMT during childhood reach adulthood and the final outcome of growth and pubertal development after BMT can be assessed. We previously reported that final height was decreased in patients who underwent BMT for haematological malignancies [4]. In the present study we investigated the effects of TBI and BMT on pubertal development and gonadal function in patients grafted for haematological malignancies during childhood.

Patients and methods

Between 1970 and 1995 148 patients suffering from haematological malignancies received an allogeneic BMT before the age of 16 years at the Department of Paediatrics, Leiden University Medical Centre. Of those patients, 79 were still alive in July 1997, 40 of whom (21 boys, 19 girls) were older than 16 years of age at the time of their last visit. These 40 patients were included in the present study, 16 of whom (10 boys) were also included in the previous study on final height [4]. Patient characteristics are given in table 1.
At the time of BMT, 25 patients were pre-pubertal. The mean age at the time of BMT was 11.3 years (range 0.9-15.9) and at the time of the last examination 19.0 years (range 16.1-25.4).
TBI was delivered by linear accelerator with energies of either 5.0 or 6.0 MV and at a midline instantaneous dose-rate of approximately 23 cGy/min.

Table 1. Patient characteristics

	Prepubertal at BMT		In Puberty at BMT*	
	Boys (n=15)	Girls (n=10)	Boys (n=6)	Girls (n=9)
Age at BMT in years:				
mean; median	9.9; 10.2	8.7; 9.1	13.8; 13.5	14.9; 14.4
(range)	(4.0 - 14.1)	(0.9 – 14.4)	(12.6 - 15.3)	(14.2 - 15.9)
Age at last visit: in years:				
mean; median	18.5; 18.2	19.1; 19.0	18.9; 18.0	19.8; 18.9
(range)	(16.1 – 21.7)	(16.5 – 23.9)	(16.9 - 23.3)	(16.8 – 25.4)
Indication:				
AML	7	7	4	6
ALL	4	1	2	2
CML	-	2	-	1
MD	2	-	-	-
NHL	2	-	-	-
TBI-dose:				
5.0 Gy	-	1	-	-
7.5 Gy	7	5	-	-
8.0 Gy	4	4	1	4
2 x 6.0 Gy	4	-	5	5
Additional Irradiation:				
Testes	2	-	-	-
Cranium	1	1	1	1

* The three male patients with uncertain pubertal status at the time of BMT are included in this category. ALL acute lymphoblastic leukaemia, AML acute myeloid leukaemia, CML chronic myeloid leukaemia, MD myelodysplasia

One girl, who underwent BMT in the 1st year of life, received a single fraction TBI of 5.0 Gy. All other patients undergoing BMT before the age of 10 years received a single fraction TBI of 7.5 Gy. Patients older than 10 years received TBI either as a single fraction of 8.0 Gy (until 1990) or as two fractions of 6.0 Gy each, on 2 consecutive days (from 1990 onwards).

Conditioning regimens included cyclophosphamide (60 mg/kg per day for 2 consecutive days) in all patients. From 1989 onwards, the patients undergoing BMT for myeloid leukaemia (n = 11) also received cytarabine (2 x 1.0 g/m2 day for 2 consecutive days) and patients with BMT for lymphoblastic leukaemia (n = 4) or non-Hodgkin lymphoma (NHL)(n = 1) received etoposide (350 mg/m2 day

for 2 consecutive days). Two boys (both treated before puberty) received an additional testicular irradiation with 10 Gy in four fractions prior to BMT; four other patients had previously received cranial irradiation with a total dose of 18 Gy in 12 fractions (n = 3) or 24 Gy in 18 fractions (n = 1).

Information about pubertal development was obtained from the medical records and consisted of the following data: Tanner stages of breast or genital development and pubic hair [5], testicular volume measured with orchidometer, age at menarche, height measurements, serum levels of LH and FSH and oestradiol in girls and testosterone in boys, data on substitution therapy with sex steroids and on the use of oral contraceptives. The onset of puberty was defined as the age at which breast development was first recorded in girls (Tanner breast stage ≥ B2) or a testicular volume ≥ 4 ml was reached in boys. If clear signs of pubertal development (e.g. penile growth and pubic hair development) had occurred in boys while testicular volume had not reached 4 ml, onset of puberty was determined on the basis of the combination of progression of Tanner stages and increasing serum levels of testosterone. When puberty was induced, the onset of puberty was defined as the start of treatment with sex steroids. In three boys puberty started approximately at the time of transplantation, but unfortunately Tanner stages and testicular volumes at the time of BMT were not recorded.

In girls puberty was induced using increasing doses of ethinyloestradiol (starting dose: 0.05 µg/kg/day). After a period of at least 1 year medroxyprogesterone was added (5 mg/kg/per day for 12 days every 4 weeks). In boys intra-muscular injections of testosterone were used (starting dose 50 mg every 3 weeks, increasing the dosages over a period of 2 years until the adult dose of 250 mg every 3 weeks was reached).

Height data were analysed for patients treated before the onset of puberty who had not received any additional irradiation (12 boys and 9 girls). Height standard deviation scores (SDS) were calculated using Dutch references [6]. Height velocity was calculated as the height increment within a time interval of 0.5-1.5 years and expressed as cm/year. Target height SDS was calculated using the formula: (maternal height + paternal height ±12 cm)/2 + 3 cm [7]. Standard laboratory methods were used for the measurement of serum levels of oestradiol, testosterone, LH and FSH (detection limits: 40 pmol/l, 0.2 nmol/l, 0.1 U/l and 0.1 U/l, respectively).

Differences in continuous variables between groups of patients were analysed with Wilcoxon rank sum tests. The Wilcoxon signed rank test was used to analyse the changes in height SDS. Associations between continuous variables were tested by Spearman correlation analyses and multiple regression analyses were used to determine the independence of the effects noted in bivariate analyses. The significance level was set at 5% in all analyses.

Results

Onset of puberty after BMT

In all boys who had not received additional testicular irradiation, puberty started spontaneously at a mean age of 13.0 years (range 10.0-15.2 years). One of these boys, treated for relapsing NHL at the age of 14.1 years, had a late onset of puberty (age 15.2 years). Hypergonadotrophic hypogonadism developed in the two boys who had received testicular irradiation and puberty was induced at the age of 14.2 and 15.3 years, respectively.

Of the ten girls treated before puberty, six (including the girl who had received cranial irradiation) had a spontaneous onset of puberty at a mean age of 11.0 years (range 9.7-12.7 years). In the remaining 4 girls puberty was induced after hypergonadotrophic hypogonadism had developed. The mean age at start of the induction therapy was 14.1 years (range 13.5-14.7 years). At the time of induction of puberty, bone age (calculated according to Greulich and Pyle) was delayed in all four girls: mean delay 2.2 years (range 1.1-3.0 years).

Progression of puberty and menarche

Puberty, as measured by progression of Tanner stages, developed normally in the 19 boys who did not receive additional testicular irradiation. At the last examination they had all reached Tanner stages G4 or more and P4 or more. Testicular volume increased above 4 ml in all boys with a spontaneous onset of puberty after BMT (range 6-14 ml), but only 7 of those 13 boys reached a testicular volume of ≥ 10 ml. The last recorded testicular volumes in the three boys with uncertain pubertal status at BMT were 12, 12 and 14 ml at the ages of 18.2, 19.7 and 16.9 years, respectively. The last recorded testicular volumes in the boys transplanted during puberty, were 8, 14 and 18 ml at the ages of

17.1, 16.8 and 16.3 years, respectively. Figure 1 represents development of testicular volumes in time after BMT. The mean testicular volume at the last measurement in all boys who had not received additional testicular irradiation was 10.5 ml (mean age 17.4 years).

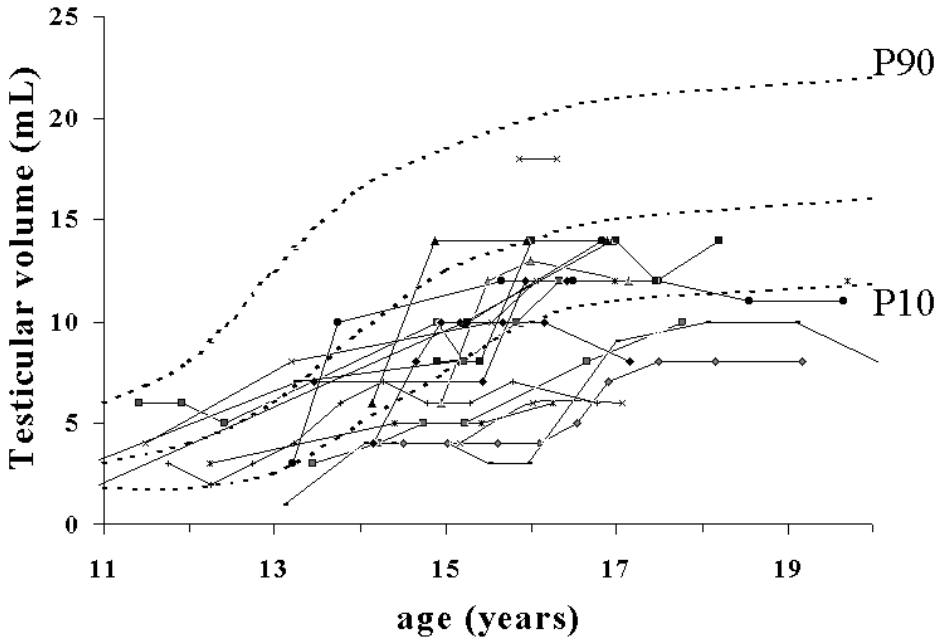

Figure 1. Testicular volume after TBI in boys who did not receive additional testicular irradiation.

The six girls with a spontaneous onset of puberty after BMT had their menarche at a mean age of 12.7 years (range 11.1-14.2 years). Of those six girls, five had a normal progression of puberty and reached Tanner stages B5 and P5 without exogenous oestrogens. The other girl had several transient

episodes of hypergonadotrophic hypogonadism, both before and after menarche. Her pubertal development was delayed and she started using oral contraceptives at the age of 16.5 years, 5 years after menarche (breast development had not progressed beyond Tanner stage B3).
All nine girls treated during puberty developed gonadal insufficiency and required hormonal substitution therapy, making it impossible to assess further spontaneous pubertal development. Before receiving exogenous oestrogens, however, one girl had regression of breast development (substitution started 3 years after BMT), and in another girl breast development had stagnated at the time substitution was started (1 year after BMT). In two of the remaining girls there were insufficient data on breast development and in the other girls substitution therapy was given within several months after BMT.

Endocrine gonadal function and fertility

In the 19 boys who had not received additional testicular irradiation, adult levels of serum testosterone (>15 nmol/l) were reached at any time after BMT (figure 2, upper panel). Episodic elevations of LH (8.1-11.5 U/l), however, were seen in 10 of them (figures 2, middle panel), and in five patients these elevations were accompanied by decreased testosterone levels (9.4-14.8 nmol/l). Elevation of FSH was found in all boys (figure 2, lower panel). In only one patient FSH levels returned to normal (<10 U/l). This patient was pre-pubertal at the time of BMT. Unfortunately, we have no data on spermatogenesis in our patients; to our knowledge none of the male patients has fathered a child.
Disturbances in the pituitary-gonadal axis were seen in all girls, even though six girls had a spontaneous onset of puberty and five of them went through puberty normally. One of these five girls (the only patient that had received a low TBI dose of 5.0 Gy) did not develop hypergonadotrophic hypogonadism, but she did show an abnormal response to a gonadotrophin releasing hormone (GnRH) test. The remaining four girls with a normal progression of puberty developed hypergonadotrophic hypogonadism, defined as elevated levels of serum gonadotrophins without detectable oestradiol. In three of them endocrine gonadal function recovered quickly without oestrogen substitution; the other girl required substitution therapy but she had already reached breast stage B5 at the time gonadal insufficiency was diagnosed (age 14.6 years). All four girls who did not require oestrogen substitution reported having regular

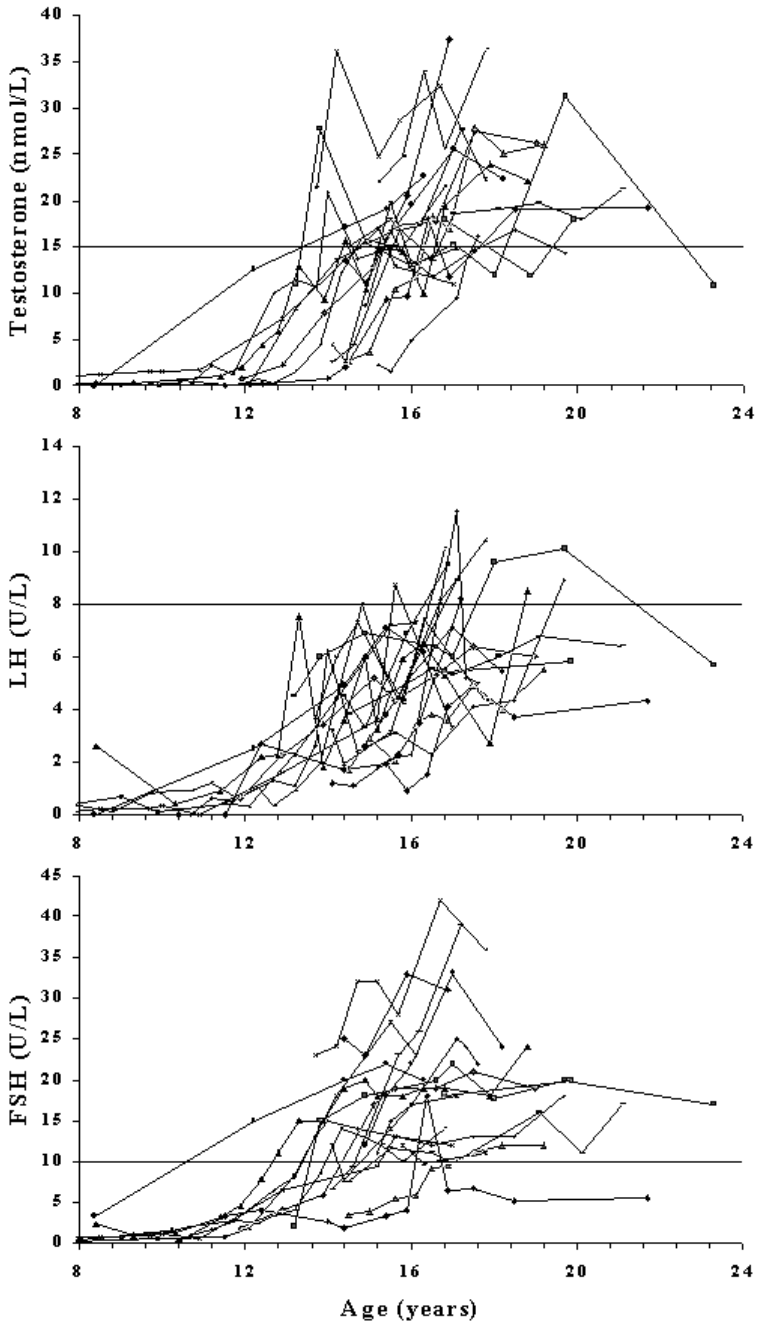

Figure 2. Serum levels of testosterone, LH and FSH after TBI in boys who did not receive additional testicular irradiation.

menses (without the use of exogenous oestrogens) during the last years of their follow up.
The remaining girls (all nine girls undergoing BMT during puberty and the four girls who did not enter puberty spontaneously) developed hypergonadotrophic hypogonadism and received substitution therapy with ethinyloestradiol and progesterone. Recovery of gonadal function after cessation of hormonal substitution was seen in one girl, who received BMT during puberty, but before menarche. This patient later became pregnant twice, both of which resulted in spontaneous abortions.

Growth

At the last examination final height (height velocity <1 cm/year) was reached by 18 of the 21 patients (12 boys and 9 girls) who were treated before puberty and had not received (additional) cranial or testicular irradiation. The other three patients had height velocities of 1.3, 1.5 and 2.1 cm/year. None of the patients were diagnosed with growth impairment due to growth hormone insufficiency or hypothyroidism. Data on (changes in) height SDS at different times after BMT are summarised in table 2.
At the last examination height SDS was significantly lower compared to target height SDS ($P < 0.001$) and compared to height SDS at BMT ($P < 0.001$); the magnitude of these differences in SDS was similar in both sexes. Height SDS also decreased between BMT and the onset of puberty ($P = 0.003$) as well as between onset of puberty and last examination ($P = 0.001$).
When analysed for boys and girls separately, the decrease in height SDS between BMT and onset of puberty was significant in girls only ($P = 0.008$), whereas the mean decrease in height SDS between onset of puberty and last visit was significant in boys only ($P = 0.002$). The total decrease in height SDS was less in the four girls who needed induction of puberty (range 0.1-1.0) than in the five girls with a spontaneous onset of puberty (range 0.9-2.1; $P = 0.05$). These two groups did not differ significantly in age at time of BMT, but the age at onset of puberty was higher in the girls who needed substitution: median 14.0 years (13.5-14.7 years) versus 11.0 years (10.6-12.7 years; $P = 0.014$).

Table 2. Height SDS and changes in height SDS in patients treated before puberty who did not receive additional irradiation

	All patients (n=21)		Boys (n=12)		Girls (n=9)	
	Mean	Median (range)	Mean	Median (range)	Mean	Median (range)
Age at onset of puberty			13.1	13.7 (10.0 to 15.2)	12.5	12.7 (10.5 to14.7)
Height SD Scores						
Target SDS	-0.1	0.3 (-1.8 to 1.1)	-0.2	0.0 (-1.8 to 0.7)	0.2	0.3 (-0.8 to 1.1)
SDS at BMT	-0.2	-0.2 (-1.8 to 1.8)	-0.4	-0.3 (-1.8 to 1.3)	0.1	0.0 (-1.0 to 1.8)
SDS at onset puberty	-0.7	-0.9 (-2.7 to 1.3)	-0.6	-0.7 (-2.0 to 1.0)	-0.7	-1.2 (-2.7 to 1.3)
SDS at last visit	-1.5	-1.7 (-3.6 to 0.9)	-1.9	-2.0 (-3.6 to -0.5)	-1.0	-1.0 (-2.2 to 0.9)
Changes in SD Scores						
from BMT until onset of puberty	-0.5	-0.5 (-1.8 to 0.4)	-0.2	-0.2 (-0.9 to 0.4)	-0.8	-0.9 (-1.8 to -0.1)
from onset puberty until last visit	-0.9	-0.9 (-3.8 to 0.9)	-1.3	-1.0 (-3.8 to -0.1)	-0.2	-0.4 (-1.2 to 0.9)
from BMT until last visit	-1.3	-1.1 (-3.6 to 0.1)	-1.5	-1.6 (-3.6 to -0.3)	-1.1	-1.0 (-2.1 to 0.1)
Difference Target SDS and SDS at last visit	-1.5	-1.6 (-3.0 to 0.3)	-1.7	-1.9 (-3.0 to 0.2)	-1.1	-1.1 (-2.5 to 0.3)

As in our previous analyses, the total loss of height SDS was more severe in patients treated at an earlier age (r = 0.76). A correlation existed, however, between the age at the time of BMT and other factors that could possibly influence final height or loss of height SDS, such as the age at onset of puberty (r = 0.76) or the TBI dose (r = 0.88). A multiple regression analysis of the factors TBI dose, age at BMT, age at onset puberty, gender and need for induction of puberty, revealed that male gender and a younger age at the time of BMT were the only factors with an independent (negative) effect on longitudinal growth.

Discussion

To evaluate the effects of TBI and BMT on pubertal development properly, patients should have completed puberty. As the BMT procedure in patients with haematological malignancies is relatively young, only recently children who have received a BMT before the onset of puberty completed their pubertal

development and reached their final height. As the prognosis for normal pubertal development and gonadal function in survivors of childhood leukaemia treated with chemotherapy alone (i.e. without BMT) is excellent [8;9], disturbances in pubertal development in survivors of BMT and TBI are likely to be the result of irradiation (e.g. total-body, testicular or cranial irradiation) or of (the additive effect of) high-dose chemotherapy used in preparative regimens. Of the hypothalamus-pituitary-gonadal axis the gonads are most vulnerable to damage caused by chemotherapy and/or irradiation; dysfunction of the hypothalamus or the pituitary gland is less common and is almost always associated with cranial irradiation, which can lead to a premature [10-12] as well as a delayed [13] onset of puberty. Our two patients who had received cranial irradiation before the onset of puberty, however, both had a normal timing of puberty.

The spontaneous onset of puberty and the subsequent normal pubertal development in all boys not receiving additional testicular irradiation is in line with the findings of other investigators [1;14;15]. The Leydig cells, responsible for the production of testosterone, are more resistant to irradiation than are spermatogonia, and recovery of Leydig cell function can be seen after absolute radiation doses as high as 24 Gy [14]. The normal pubertal development and the fact that all patients had reached adult serum levels of testosterone at some time after BMT, however, do not necessarily mean that Leydig cells are unaffected. The elevated LH and decreased testosterone levels, incidentally found in the serum of some patients, suggest that TBI can cause subtle changes in Leydig cell function. Other investigators have found decreased responses to HCG stimulation after TBI [3], which further supports this suggestion.

The elevated levels of FSH in all boys and the relatively small testicular volumes indicate that the germinal epithelium of the testis is damaged by the preparative regimens used. In contrast to others [2;16] we did not find a trend towards normalisation of FSH levels in our male patients, even though most patients received single fraction irradiation which is believed to be less detrimental to the testicular germinal epithelium than is fractionated irradiation. This can be explained by the lower total radiation dose used in single-fraction TBI, but there is also evidence that fractionation of one dose may lead to longer sterile periods in man [17]. Variations in the exposure to alkylating agents prior to BMT could be responsible for this absence of testicular recovery, as the

cumulative dosage rather than the daily dosage is the most important factor determining gonadotoxicity of cyclophosphamide, also used in the preparative regimens [16;18;19]. In view of the known vulnerability of germinal epithelium to irradiation and the elevated FSH levels, we expect most of the male patients to be infertile. We have no data on semen analysis in our patients, however, and recovery of spermatogenesis has been described 7 to 8 years after a single fraction of 10 Gy TBI [2]. Moreover, in a group of 463 male TBI patients, Sanders et al. [2] found evidence of testicular recovery in 17% (defined as normal serum levels of LH, FSH and testosterone with evidence of sperm production), and 5 of these patients had fathered one or more children. There was no evidence for increased risk of congenital malformations or complications during pregnancy or delivery. Ovarian damage can be caused by alkylating agents as well as by irradiation. As a total dose of at least 20.0 g of cyclophosphamide is required to produce amenorrhoea in women younger than 30 years of age [2], it is likely that most of the disturbances found in our patients can be attributed to the irradiation and not to the cyclophosphamide used in the conditioning (120 mg/kg).

Gonadal function was less often affected in girls treated before the onset of puberty compared to those treated during puberty. The percentage of girls treated before puberty with a spontaneous onset of puberty and menarche (60%) is comparable with that reported by Sarafoglou et al. (56%) [15], even though their patients had received hyperfractionated TBI. Others have reported slightly lower incidences of spontaneous onset of puberty after TBI between 31% and 45% [15;20]. The finding that all girls treated after the onset of puberty developed gonadal failure is also in line with reports of other investigators [21-23] and suggests that the pre-pubertal ovary is more resistant to irradiation. Attempts to limit radiation-induced damage to the (post-) pubertal ovary using GnRH analogues have been successful in rats [24], but not in rhesus monkeys [25]. As radiation doses used in these animal studies (1 x 30 Gy in rodents and 20 x 2 Gy in monkeys) are higher than those used in TBI, it would be worthwhile to investigate whether the use of GnRH analogues can reduce ovarian damage in patients receiving TBI after the onset of puberty. Recovery of ovarian function occurred in one girl, transplanted for acute myeloid leukaemia (AML) during puberty. She became pregnant twice, but unfortunately both pregnancies resulted in spontaneous abortions. Women receiving high-dose alkylating agents and TBI are at risk for spontaneous

abortions, pre-term delivery and low birth weight infants, as described by Sanders et al. [26]. In this large survey, female TBI patients had a spontaneous abortion rate of almost 40% and the incidence of pre-term labour and delivery was >60%. This is probably caused by radiation-induced changes in the myometrium or uterine vasculature [26;27]. If gonadal function is preserved or recovers, however, patients are still at risk for early menopause [28], as is shown by the girl who had a spontaneous onset of puberty and menarche but later developed secondary amenorrhoea and hypogonadism. As recovery of ovarian function after hypergonadotrophic hypogonadism is possible, hormonal substitution should be stopped at regular intervals in order to re-evaluate gonadal function.

It should be emphasised that patients should be well-informed about the risk of infertility as well as the possibility of (recovery of) fertility and the increased abortion rate in order to allow them to make appropriate decisions about procreation and contraception. In children treated during puberty the possibility of germ cell preservation should be discussed with patients and/or parents.

The use of TBI in preparative regimens for BMT has an adverse effect on growth and final height [4;29;30]. Potential mechanisms include radiation-induced hypothyroidism, growth hormone deficiency and damage to the epiphyseal growth plate [3]. The results of the present height analyses are comparable to the results we reported previously [4]. In this previous study, pre-pubertal linear growth velocity SDS was not statistically different from zero in the first 3 years after BMT. In the present analyses we compared height SDS at the time of BMT to height SDS at the onset of puberty. In all girls and most boys, height SDS at the onset of puberty was lower than at the time of BMT suggesting that loss of height SDS already occurred before the onset of puberty, even when puberty was not delayed. The fact that decrease in height SDS was greater when patients were treated at a younger age, (a result which was found in a European study on final height after BMT as well [31]) further supports the suggestion that height SDS already decreases before the onset of puberty in these patients.

Although there was no significant difference between boys and girls in the decrease in height SDS, in a multiple regression analysis, male gender (but not oestrogen substitution) proved to be an independent factor determining this decrease. Interestingly there was a difference in total loss of height SDS since transplantation between girls who had a spontaneous onset of puberty and

girls who needed induction of puberty. The most likely explanation for this is that, in the case of induction of puberty, the relatively late introduction of relatively low doses of oestrogens had resulted in a prolonged period of growth by delaying progression of bone age, thereby increasing final height. All four girls in whom puberty was induced did indeed have a delay in bone age at the onset of puberty.

We previously suggested that a lack of compensatory increase in peak linear growth velocity, an increase that is present in normal early-developing children, could be responsible for a correlation between age at onset of puberty and decrease in height SDS [4]. Although this is a plausible explanation, we cannot exclude that the correlation between height loss and timing of puberty is caused by selection bias, as only those patients who were not in puberty at the time of BMT were included in the previous (and present) analyses of growth. This way a correlation was created between the age at onset of puberty and the age at BMT, as patients who entered puberty early could only be included if they were treated at a younger age, whereas patients treated at a relatively later age could only be included if they entered puberty late. In the multiple regression analysis the age at onset of puberty did not prove to be an independent factor determining the decrease in height. Therefore, more data are necessary in order to establish the influence, if any, of the timing of puberty on the final height of patients receiving BMT and TBI.

After TBI and BMT for haematological malignancies, pubertal development is normal in most boys, although subtle changes in Leydig cell function can be expected and spermatogenesis is likely to be severely affected. In approximately 50% of girls treated before puberty, pubertal development (and gonadal function) is normal, whereas gonadal insufficiency can be expected in the vast majority of those girls treated after the onset of puberty. As changes in gonadal function are possible and data on long-term prognosis for fertility and endocrine gonadal function are limited, careful follow up remains necessary.

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PATTERNS OF GROWTH AND BODY PROPORTIONS AFTER TOTAL-BODY IRRADIATION AND HAEMATOPOIETIC STEM CELL TRANSPLANTATION DURING CHILDHOOD

Pediatric Research 2006;59:259-264

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Abstract

Patterns of growth and body proportions were studied in 75 children receiving total-body irradiation (TBI) and haematopoietic stem cell transplantation (SCT) before onset of puberty. Of the 19 patients receiving growth hormone (GH), only data obtained before onset of GH were included.

Thirty-two patients reached final height (FH). Median change in height SD score (SDS) between SCT and FH was -1.7 in boys and -1.1 in girls.

Peak height velocity (PHV) was decreased in the majority of the patients (median PHV 5.7 cm/yr in boys and 5.3 cm/yr in girls), even though it occurred at appropriate ages.

Changes in body proportions were analysed by linear mixed-effects models. Decrease in sitting height SDS did not differ between boys and girls (0.15 SD/yr). In boys, decrease in leg length SDS was of comparable magnitude (0.12 SD/yr), whereas in girls decrease in leg length was less pronounced (0.02 SD/yr), leading to a significant decrease in SDS for sitting height/height ratio in girls only.

The sex-specific effects of several variables (e.g. age at SCT, time since SCT, onset of puberty) on height SDS were analysed by linear mixed-effects modelling, showing a slightly faster decrease in younger children, and a more pronounced decrease during puberty in boys compared with girls.

We conclude that 1) younger children are more susceptible to growth retardation after TBI and SCT, 2) pubertal growth is more compromised in boys, and 3) leg growth is relatively less affected in girls, possibly due to a high incidence of gonadal failure in girls.

Introduction

Haematopoietic stem cell transplantation (SCT) has become a standard treatment option for many children with congenital or malignant disorders of the haematological system. The intensive conditioning regimens required in most cases, often result in impaired growth and reduced final height, especially if unfractionated total-body irradiation (TBI) is part of the conditioning regimen [1]. TBI can impair growth both directly, by damaging the epiphyseal growth plates, and indirectly, by decreasing growth hormone (GH) secretion or by causing hypogonadism or hypothyroidism [2]. Other factors that may contribute to growth delay are chronic graft-versus-host disease in recipients of allogeneic transplants, use of corticosteroids, psychosocial dysfunction, and insufficient nutritional intake.

In contrast to the extensively documented negative effect of TBI and SCT on height, more recently completed with data on final height [1;3-8], the effect of TBI and SCT on other aspects of growth are less intensively studied. To our knowledge, for example, height development after TBI and SCT (including influences of sex and puberty) has not yet been modelled, and the effect on body proportions has been subject of only few studies [5;9-12]. Moreover, most of these studies included patients who had received cranial irradiation prior to TBI, patients who had already entered puberty at the time of SCT or patients who had been treated with GH. In addition, none of these studies considered sex differences.

In an attempt to clarify these aspects of growth after SCT, we investigated sex-specific development of height, body proportions and final height in children receiving TBI and SCT for haematological malignancies before the onset of puberty. Patients who had received cranial irradiation were excluded, as well as height measurements taken after onset of GH therapy. In addition, we constructed sex-specific models of growth after TBI and SCT.

Patients and methods

Between 1980 and 2001, 193 prepubertal children with a haematological malignancy received TBI-based conditioning for SCT at the Leiden University

Medical Centre. All children without a relapse after SCT, who had more than 2 years of follow-up after TBI-based conditioning for SCT for a haematological malignancy before the onset of puberty were selected for this study (n= 89). We excluded patients who had received cranial irradiation (n= 6) or had developed chronic graft-versus-host disease (n=7) or had Down syndrome (n=1). A total of 75 patients were included; their characteristics are summarised in table 1. Puberty onset had occurred in 61 patients (40 males).

Table 1. Population characteristics.

	Male	Female
Number of patients	53	22
Age SCT	8.2 (2.3 to 13.9)	7.2 (0.8 to 12.3)
Age puberty (40 m / 21 f)	12.3 (9.8 to 14.5)	10.9 (9.0 to 14.1)
Follow-up (in years)	8.4 (2.0 to 15.5)	11.0 (3.5 to 20.2)
Gonadal failure < age 15 yr	8	11
SCT Type		
Autologous	4	2
Identical Related	37	16
Matched unrelated	8	3
Haplo-identical	4	1
Diagnosis:		
ALL	26	7
AML	18	12
MDS	5	2
NHL	2	1
CML (Ph+)	2	-
TBI dose		
5.0 Gy	-	2
7.0 Gy	8	3
7.5 Gy	26	12
8.0 Gy	7	2
2x6.0 Gy	12	3
Testicular booster 4x2.5 Gy	8	n.a.

Serum level of Insulin-like Growth Factor 1 and Insulin-like Growth Factor binding protein 3 were measured annually in all patients, and patients were tested for GH deficiency if growth was impaired. GH therapy was given to 19 patients (13 males). Indications for GH therapy were GH deficiency (n=1), GH neurosecretory dysfunction (n=2) and growth impairment (n=16). Only data obtained before start of GH therapy were included.
The study was approved by the by the Institutional Review Board (Leiden University Scientific Review Board) and the Review Board of the Netherlands' Organization for Scientific Research (NWO).

Conditioning for SCT

Conditioning for SCT consisted of TBI and cyclophosphamide in all patients. In addition to cyclophosphamide (60 mg/kg/day i.v. for 2 consecutive days), cytarabine (1 g/m^2/day for 2 consecutive days) was given to patients treated for myeloid leukaemia or MDS between 1988 and 1998 (n=20). From 1990 onward, patients treated for lymphoblastic leukaemia or non-Hodgkin lymphoma (n=32) received etoposide (350 mg/m^2/day for 2 consecutive days) in addition to cyclophosphamide. All patients received unfractionated TBI, delivered at a mean dose rate of 25 cGy/min. As age is an important determinant of the tolerable total irradiation dose in children, a TBI regimen with age-dependent total dose was applied (0-2 years: 5.0 Gy, 2-4 years: 7.0 Gy, 4-10 years: 7.5 Gy, >10 years 8.0 Gy). The latter dose was 'increased' in 1989 to two single fractions of 6.0 Gy, given on 2 consecutive days (instead of the equivalent 9.0 Gy once, which had too many side effects in adults). Eight boys received additional prophylactic testicular irradiation (10 Gy in four fractions prior to TBI).

Auxological parameters

Data on height, sitting height, Tanner stages of breast or genital development [13] and testicular volume (measured with an orchidometer) were collected from the clinical records. Sub-ischial leg length was defined as height minus sitting height. The ratio between sitting height and height was used as parameter for body proportions. Auxological parameters were expressed as standard deviation scores (SDS) for sex and age, all based on the same reference population (n=14.500) from the1997 Dutch Growth Study [14;15]. Patients were

considered to have reached final height if height velocity was less than 1 cm/year in pubertal subjects.

Puberty and gonadal function

The onset of puberty was defined as the age at which breast development was first recorded in girls (Tanner breast stage ≥ B2) or a testicular volume ≥ 4 mL was reached in boys. If signs of pubertal development (e.g. penile growth and pubic hair development) had occurred in boys while testicular volume had not reached 4mL, onset of puberty was determined on the basis of the combination of progression of Tanner stages and increasing serum levels of testosterone. If puberty was induced, onset of puberty was defined as the onset of sex hormone replacement therapy. In girls, hypergonadotrophic hypogonadism was used as a parameter of ovarian failure. In boys, elevated serum levels of luteinising hormone (LH) with or without decreased serum levels of testosterone were used as a parameter for Leydig cell failure, and elevated levels of follicle stimulating hormone (FSH) were used as an indicator of Sertoli cell failure (as a result of germ cell loss and/or Sertoli cell loss).

Statistical analyses

S-PLUS 6 Professional (Insightful Corp., Seattle WA, USA) was used for all statistical analyses, with significance level set at 5%.

A height velocity curve was fitted by monotone smoothing procedure as described by Ramsay et al. [16;17] for each individual who had at least five height measurements between 8 and 16 years of age for girls (n=16) or 10 and 18 years of age for boys (n=29), and peak height velocity (PHV) and age at PHV were calculated. We used penalized least squares with a penalty that depended on the number of measurements (penalty $250/N^2$, with N = the number of individual measurements).

Changes in SDS for the different auxological measurements with time since transplantation were fitted by mixed-effects models with data grouped by individual patients, SDS as dependent variable and time since SCT as major independent variable (see next paragraph for background information on mixed-effects models). We used the function ‘lme’ (linear mixed-effects) in S-PLUS.

Choice of analysing methods

The analysis of growth after SCT is a typical example of a repeated measurements analysis, in which both the number of observations and the interval between the observations may vary between the patients (i.e. both time between visits and duration of follow-up may vary). A traditional standard linear regression analysis assumes all measurements to be independent and may yield biased results, especially if the data are unbalanced. An alternative is to fit a growth curve separately for each individual and average the patterns. This has been done for the height velocity analyses, but has the disadvantage that estimated patterns are very uncertain for individuals with few measurements. Therefore, we only used data of patients with at least five measurements in the pubertal age for the construction of the pubertal height velocity curves.
Mixed-effects models also fit a growth curve for each individual, after which the parameters for the growth curves are averaged to obtain a population effect. However, for persons with relatively little information, information is used from the other individuals to obtain their growth curve parameters. This is obtained by assuming that the growth curve parameters are a random sample from some distribution (usually a normal distribution), and the parameters are included as 'random effects'. Moreover, the effects of some covariates can be chosen to be equal for all individuals, and these covariates are only included as 'fixed effects'. The use of mixed-effects models (combining random and fixed effects) allows the inclusion of all available data in the analyses without the risk of considerable bias. As far as we know, mixed-effects models have not been used before in the analysis of growth after SCT.

Identification of factors influencing height SDS after SCT in the mixed-effects model

We used time, sex, puberty, age at SCT and gonadal failure as independent variables and height SDS after SCT as dependent variable for our model. The basis of our model was a linear change (decrease) in height SDS with time since SCT. As patients were recovering from their illness, we allowed the slope to be different in the first two years after SCT compared with the following years. The value of the intercept and the slopes in the first two years after SCT and in the years thereafter differed per individual, as was expressed by their random effects. The slope of the major time variable was allowed to depend on

age at SCT, sex and the presence of gonadal failure before the age of 15 years. The effect of gonadal failure was allowed to depend on sex.
The effect of puberty on height SDS was divided into two components. The first component is a reference population effect: when patients from the same sex reach the pubertal age (approximately 8.5 years in girls and 10.5 years in boys) height velocity of the reference population gradually increases as a result of the pubertal growth spurt and subsequently decreases. If the child itself has not yet reached puberty, this will cause its height SDS to decrease in a non-linear way. The second component of the effect of puberty is the individual pubertal growth spurt of our patients, which will result in a gradual increase in height SDS after onset of puberty, which stabilises after 3 to 5 years. In patients with an early onset of puberty height SDS may even decrease at the end of their growth spurt. As this second component of puberty is an individual effect, which may vary between patients, it was included as a random effect as well. We allowed both puberty effects to depend on sex. We used sigmoid curves (logit transformations) to model both puberty effects.

Formula 1 represents the relation for the sigmoid curve of the population effect, with x_1 being the time in years since reaching the pubertal age (8.5 in girls and 10.5 in boys).

Formula 1: $$\beta 1\left(\frac{e^{(x_1 \cdot scale-2)\cdot 2}}{1+e^{(x_1 \cdot scale-2)\cdot 2}}\right)-0.01798621$$

Formula 2 represents the relation for the sigmoid curve of the individual puberty effect, with x_2 being the time since individual onset of puberty in years.

Formula 2: $$\beta 2\left(\frac{e^{(x_2 \cdot scale)}}{1+e^{(x_2 \cdot scale)}}\right)-0.5$$

The second part of both formulas is a correction forcing the results to equal zero at x=0. The optimal scale parameter, which is included in the model in a non-linear way, was obtained by fitting the model for an array of values of

'scale'. The combination with the best Bayesian Information Criterion (BIC) value was chosen.

Results

Height and height velocity

A total of 974 height measurements from 75 individuals contributed to the results. Figure 1 represents the smoothened individual curves of height velocity of 29 males and 14 females.

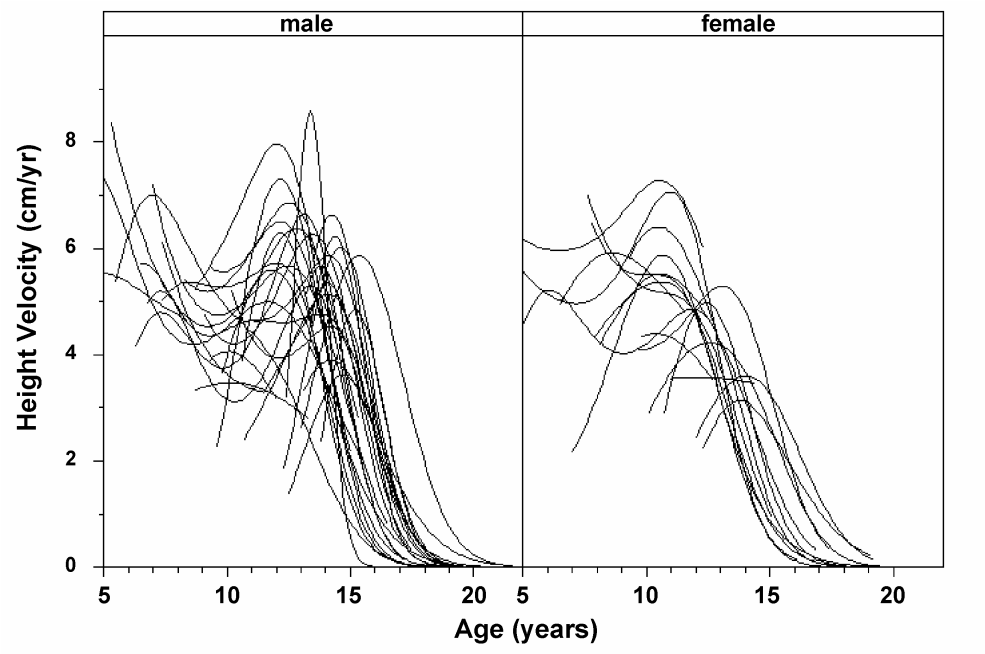

Figure 1. Smoothened individual curves for height velocity of males (n=29) and females (n=14).

In 4 patients (3 girls) no pubertal growth spurt could be detected; all showed an attenuation of decrease in height velocity between 10.0 and 12.5 years of age. Of the patients with a detectable pubertal growth spurt, median pubertal PHV was 5.7 cm/year in boys (n=29, range 3.5 – 8.6 cm/year) and 5.3 cm/year in girls (n=14, range 3.1 – 7.3 cm/year). Median age at PHV was 13.4 years

(10.1 – 15.4) in boys and 10.9 years (8.6 – 14.0) in girls. Final height (FH) was reached by 32 (21 males) of the 56 patients who had not received GH. Of the patients who had reached FH, age and height SDS at time of SCT, at onset of puberty and at final height are summarised in table 2.

Table 2. Age and height SDS at different times between SCT and FH of the 32 patients who have reached final height.

	Male (n=21)		Female (n=11)	
Age (years)				
SCT †	10.9	(5.6 to 13.9)	8.2	(0.8 to 12.3)
puberty onset *	13.2	(10.0 to14.5)	11.2	(10.0 to 14.1)
final height *	18.0	(15.4 to 20.2)	17.4	(16.0 to 19.2)
Time (years)				
SCT to puberty onset	1.5	(0.3 to 5.6)	2.8	(1.2 to 9.7)
puberty onset to final height	5.6	(2.6 to 7.3)	5.1	(3.9 to 7.8)
Height (SDS)				
target height	+0.4	(-1.7 to +1.3)	-0.2	(-0.5 to +0.9)
SCT	-0.6	(-1.9 to +0.9)	-0.6	(-1.9 to +1.5)
puberty onset	-0.8	(-2.3 to +0.9)	-1.4	(-2.4 to +1.1)
final height	-1.8	(-3.7 to +0.1)	-1.6	(-3.1 to +0.5)
Height (cm)				
target height §	186.9	(171.7 to 193.0)	169.5	(167.1 to 176.3)
final height **	171.0	(157.6 to 184.4)	159.9	(150.7 to 173.8)
Height differences (SDS)				
puberty onset - SCT *	-0.1	(-1.1 to +0. 7)	-0.5	(-1.2 to +0.0)
final height - SCT	-1.7	(-3.4 to -0.0)	-1.1	(-2.2 to -0.0)
final height - target height	-2.1	(-4.5 to -0.0)	-1.4	(-2.8 to +0.1)
Height differences (cm)				
final height - target height	-14.9	(-32.1 to -0.2)	-9.3	(-18.2 to +0.4)

Data represented as median (range). Patients treated with GH are excluded from analyses.
Significant differences between boys and girls (Wilcoxon rank sum test) are marked by symbols: †=p=0.051; *= p<0.05; **=p<0.01; §=p<0.001.

Body proportions

Since 1991, sitting height was measured on a routine basis as part of the follow-up protocol. A total of 373 sitting height measurements from 69 patients (19 females) contributed to the results. The results of the linear mixed-effects models for changes in SDS with time since SCT for the different auxological measurements are summarised in table 3.

Table 3.
Results of linear mixed-effects models for changes in SDS with time since SCT.

	Intercept ¶	Change in SDS per year since SCT	
		Males (n=50)	Females (n=19)
Height	- 0.482 *	- 0.136 **	- 0.112 **
Sitting height	- 0.145 §	- 0.147 **	-0.149 **
Leg length	- 0.609 **	- 0.117 **	- 0.021 §
Sitting height : height	-0.463 **	+ 0.002 §	- 0.094**

¶ The intercepts did not differ between males and females
§ Not significant; * p<0.001; ** p<0.0001

In males, there was a significant decrease in both sitting height SDS and leg length SDS, and these changes were of comparable magnitude. Therefore, the SDS for the sitting height/height ratio did not change. In females, however, the significant reduction in sitting height SDS was not accompanied by a significant reduction in leg length SDS, resulting in a significant decrease in sitting height/height ratio SDS. The decrease in sitting height/height ratio SDS was more pronounced in the 10 girls with ovarian failure before the age of 15 years (estimate -0.117 SD/year; p<0.0001; standard error 0.017) compared with the 9 girls without ovarian failure (-0.032 SD/year; p=0.49; standard error 0.046). In boys, no differences were detected between the 8 boys with Leydig cell failure (all had received additional testicular irradiation) and the 42 boys without Leydig cell failure.

Model for changes in height SDS after SCT

The final model is summarised in table 4 and the resulting curves are represented in figure 2.

Table 4. Summary of the model of change in height SDS after TBI (n=75).

	Value	95% CI	p-value
Height SD at transplant	-0.49	-0.72 to -0.26	<0.0001
Time since SCT male	-0.13	-0.17 to -0.084	<0.0001
idem female to male*	-0.031	-0.093 to 0.03	0.3211
Slope deviation first 2 years after SCT	0.0000018	-0.056 to 0.056	0.9999
Age at SCT (from 4 years onward)	0.009	0.0022 to 0.016	0.0100
Change slope at onset of puberty in reference population male	0.062	0.0089 to 0.11	0.0227
idem female to male*	0.014	-0.056 to 0.085	0.6887
Time since onset puberty reference population (β1 in formula 1) male§	-1.9	-2.2 to -1.6	<0.0001
idem female to male*	0.67	0.22 to 1.1	0.0039
Time since onset puberty individual patient (β2 in formula 2) male§	0.72	0.23 to 1.2	0.0047
Idem female to male*	0.45	-0.34 to 1.2	0.2702

All time parameters are expressed in years. Overall p-values for effects of time since transplant, onset of puberty in the reference population, onset of individual puberty and sex were all <0.0001. The overall effect of sex on population start puberty had p=0.0007.

*: Difference between females and males.

§: See figure 2 for visualisation of effects.

The model describes a linear decrease in height SDS with time after SCT. This decrease did not differ significantly (p=0.32) between boys and girls (figure 2a and 2d). For boys receiving SCT at the age of four, the decrease per year was -0.13 SD/yr (p<0.0001), whereas for girls it was –0.16 SD/yr. For each year increase in age at SCT, this decrease in height SDS is 0.009 SD/yr less (p=0.01). The effect of reaching the population pubertal age (i.e. 8.5 years in girls and 10.5 years in boys) was best described by a combination of a linear

increase in height SDS (0.062 SD/yr for males, 0.076 for females) and a logistic decrease in height SDS with β1 = -1.9 for males and –1.2 for females, and scale parameter 0.4 (see formula 1 and figure 2b and 2e). The decrease in height SDS caused by reaching the pubertal age was significantly greater in boys compared with girls (p=0.0007). A modest pubertal growth spurt could be detected in both boys and girls, with a logistic increase in height SDS after onset of puberty with β2 = 0.72 for males and 1.17 for females, and scale parameter 0.8 (see formula 2, figure 2c and 2f). The difference in growth spurt between boys and girls was not significant (p=0.27).

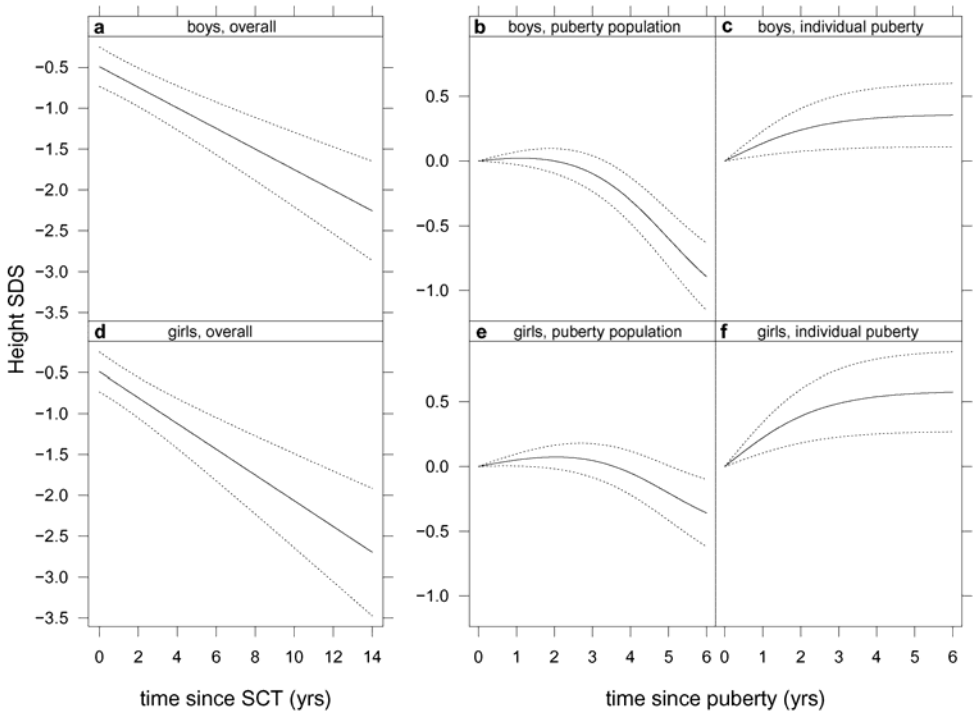

Figure 2. Graphic representation of several components of the model for growth after TBI-based conditioning for SCT, based on all 75 patients (dotted lines represent the 95% confidence intervals). The upper three panels (a,b,c) represent the males, the lower three panels (d,e,f) females. The panels on the left (a,d) represent change in height SDS with time since SCT. The panels in the middle (b,e) represent change in height SDS with time since onset of puberty in the reference population (10.5 years in boys and 8.5 years in girls). The panels on the right (c,f) represent changes in height SDS with time since the individual onset of puberty.

Total TBI dose was determined by age at SCT. The effect of age at SCT was opposite to and dominant over the effect of total TBI dose. Therefore, total TBI dose was not included in the final model. 'Gonadal failure' was not included in the final model, as it did not significantly improve the fit.

Discussion

The present study is one of the largest single-centre study to date on growth and final height after TBI-based conditioning for SCT. In addition, it is the first study to analyse the effects of unfractionated TBI on body proportions in boys and girls separately, thereby identifying sex-differences in development of body proportions after single dose TBI. By using the mixed-effects model approach, we were able to identify several factors that influence growth after TBI-based conditioning for SCT.

Pubertal growth spurt

Reference data on height velocity in Dutch children are not available. Beunen and Malina [18] summarised the results of 20 studies on pubertal PHV, in which mean PHV ranged from 8.2 to 10.3 cm/year for males and from 7.0 to 9.1 cm/year for females. The mean age at PHV in these studies ranged from 13.4 to 14.4 in males and from 10.2 to 12.6 in females. Although our patients reached PHV at appropriate ages, PHV was lower in the vast majority of them. We therefore conclude that the pubertal growth spurt is blunted in our patients. In the patients who had reached final height, the age at PHV was only slightly higher than the age at onset of puberty. As patients visited the clinic only once or twice a year, there might have been a delay of up to 12 months in the appreciation of onset of puberty by clinical definition. In addition, testicular volume as indicator of onset of puberty may be less suitable in our patients, as testicular volume is compromised after TBI [6].

Final height

Of the 75 patients included in the present study, 32 had reached final height without receiving GH therapy. The decrease in height SDS between TBI and final height in these patients was 1.1 SD in girls and 1.7 SD in boys, which is comparable to that described in most studies on final height after SCT (i.e. 1.0

- 1.5 SD) [1;3;4;7;8]. Sf-TBI is generally believed to have a much greater impact on growth than does fractionated TBI (f-TBI) [19]. This belief is largely based on studies in patients receiving higher doses (9-10 Gy) sf-TBI, and these studies did not include final height. Recently, several single centre studies reported final height data after TBI in prepubertal patients who had not received additional cranial irradiation. Frisk et al. used 7.5 Gy sf-TBI and reported a median decrease in height SDS of 1.1 SD in 9 patients [7]. Cohen et al. used 12 Gy f-TBI and reported a mean decrease in height SDS of 0.9 SD in 14 patients [4]. Sanders et al. used 12 to15.75 Gy f-TBI and report a mean decrease in height SDS of approximately 1.5 SD in 21 patients receiving TBI before the age of 10 years [8]. The only multi-centre study by Cohen et al. report similar final height SDS in 39 pre-pubertal children receiving sf-TBI (-1.2 ±1.1 SD) compared with 39 children receiving f-TBI (-1.0 ± 1.2 SD), although total decrease in height SDS was slightly greater in the patients receiving sf-TBI (1.4 versus 0.9 SD) [1]. Based on the results of the available final height data, we conclude that sf-TBI and f-TBI may have a similar effect on growth (i.e. mean decrease in height SDS after pre-pubertal TBI of approximately 1 to 1.5 SD).
As in previous studies, decrease in final height was greater in boys (median 1.7 SD versus 1.1 SD in girls). In addition, in boys most of the decrease in height SDS occurred during puberty, whereas in girls decrease in height SDS was slightly greater before puberty (not significant) and much less during puberty (all puberty-sex interactions, with boys as the reference category, were positive). There are several possible explanations for these differences between boys and girls. First, the time between SCT and onset of puberty was slightly greater in girls (median 2.8 years versus 1.5 years in boys). Second, it is possible that maximum height velocity is limited by radiation-induced structural damage to the growth plates (e.g. to 5-6 cm/year). As PHV is greater in healthy boys compared with girls, limiting the PHV may have had a greater effect on boys. Third, ovarian failure frequently occurred in girls, whereas Leydig cell failure only occurred in the 8 boys who received a testicular booster irradiation. Delayed introduction of sex hormone replacement therapy in girls may have resulted in a prolonged period of prepubertal growth.

Body proportions

Data on body proportions after TBI-based conditioning for SCT are limited. Three studies compared sitting height SDS to leg length SDS [9-11], two other studies compared sitting height SDS to standing height SDS [5;12]. All studies

describe a greater impairment of growth of the spine compared with that of the lower limbs, with differences between sitting height SDS and height (or leg length) SDS of 0 SD to -1.5 SD (on average). Most studies, however, included a relatively small number of patients, or patients who had received cranial or craniospinal irradiation, or were treated with growth hormone [5;9-11]. In addition, none of the previous studies investigated the differences in body proportions between boys and girls.

In our study, changes in SDS for height, sitting height, leg length and sitting height/height ratio were investigated in boys and girls separately. In boys, the decreases in SDS for sitting height and leg length were of comparable magnitude. Therefore, sitting height/height ratio SDS did not change. In girls, however, a decrease in sitting height SDS was not accompanied by a decrease in leg length SDS, resulting in a significant decrease in sitting height/height ratio SDS. As oestrogen deficiency causes a relative increase in leg length compared with sitting height, we hypothesise that lack of oestrogen is responsible for the decrease in sitting height/height ratio SDS in girls. Indeed, the decrease in sitting height/height ratio SDS was more pronounced in girls diagnosed with gonadal failure before the age of 15 years. Possible explanations for the differences between our results and those of previous studies are the separate analysis of boys and girls, the exclusion of patients with a history of CNS irradiation or GH treatment, and differences in TBI regimens (most of our patients received a lower total dose of TBI (7-8 Gy sf-TBI) compared with the other studies (9-10 Gy sf-TBI or >10 Gy f-TBI).

Modelling growth after SCT

Modelling growth after SCT is complicated by the fact that there are several time variables to consider (i.e. chronological age, time since SCT and time since onset of puberty), all of which are closely correlated. As we were interested in the effects of TBI and SCT on growth, we chose the SCT as starting point, with time since SCT as our primary time variable. As changes in absolute height in normally growing children are influenced by chronological age, we chose changes in height SDS as our main outcome variable. The effect of puberty on changes in height SDS was separated into the effect of the pubertal growth spurt in the reference population and the effect of the patients' individual pubertal growth spurt (as mentioned in the methods). On empirical basis, we have used non-linear effects to account for these different effects of puberty. The other parameters were added to the model as linear effects. The

parameters ‘time since SCT’ and ‘individual puberty’ were added to the model with both fixed and random effects. The final model shows that in our patient population, there is a constant decrease in height SDS with time after TBI-based conditioning for SCT, with no significant sex difference. It also shows a slight attenuation of this constant decrease with increasing age at SCT, even though the TBI dose was lower in younger children. This suggests that younger children are more vulnerable to the growth limiting effects of radiation. During puberty, the increase in height SDS as a result of the individual pubertal growth spurt of our patients was slightly greater in girls compared with boys (figure 2c,f), but the difference was not significant. In boys (but not in girls), the individual pubertal growth spurt could not compensate for the loss of height SDS caused by the pubertal growth spurt in the reference population, leading to an accelerated loss of height SDS during puberty. As the logistic component of the negative effect of the pubertal growth spurt in the reference population was greater in boys compared with girls, the greater loss of height SDS during puberty in boys was more likely to be the result of the greater pubertal growth spurt in the reference population than to a lower individual growth spurt in the boys in our study. Indeed, median pubertal PHV after SCT was slightly higher in boys compared with girls after TBI, but the difference between the sexes (0.5 cm/year) was less than observed between boys and girls in the normal population (>1 cm/year).

This study shows that growth and body proportions after TBI and SCT are affected in a sex-specific way. It identifies several influencing factors that may help to clarify mechanisms behind growth delay after TBI and SCT. Moreover, this model of growth after TBI provides us with a powerful tool for future evaluations of the effect of growth hormone treatment on growth after TBI.

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GROWTH HORMONE (GH) SECRETION AND RESPONSE TO GH THERAPY AFTER TOTAL-BODY IRRADIATION AND HAEMATOPOIETIC STEM CELL TRANSPLANTATION DURING CHILDHOOD

Submitted for publication

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Abstract

Growth is often impaired in children receiving total-body irradiation (TBI) as conditioning for haematopoietic stem cell transplantation (SCT). Radiation damage to the growth plates is an important cause, but decreased growth hormone (GH) secretion may also play a role. In January 1997 we introduced a protocol for the treatment with GH of children with impaired growth after single-fraction TBI. This study is an evaluation of that protocol. The main outcome measure is the effect of GH therapy on height SDS after onset of GH therapy, estimated by random-effect modelling with corrections for sex, age at time of SCT and puberty (data analysed on intention-to-treat basis). Between January 1997 and July 2005, 66 patients (48 male) treated for haematological malignancies had at least two years of disease-free survival after TBI-based conditioning for SCT. Stimulated and/or spontaneous GH secretion was decreased in 8 of the 29 patients tested because of impaired growth. Treatment with GH (daily dose 1.3 mg/m2 body surface area) was offered to all 29 patients and initiated in 23 of them (17 male). At time of analysis, median duration of therapy was 3.2 years; median follow-up after start of GH therapy was 4.2 years. The estimated effect of GH therapy, modelled as non-linear (logit) curve, was +1.1 SD after 5 years. Response to GH therapy did not correlate to GH secretion status.

We conclude that GH therapy has a positive effect on height SDS after TBI, irrespective of GH secretion status.

Introduction

Growth impairment is a frequent complication in children receiving total-body irradiation (TBI)-based conditioning for haematopoietic stem cell transplantation (SCT), with a mean decrease in height SDS between pre-pubertal SCT and final height of approximately 1.0 to 1.5 SDS in most studies [1-5]. Radiation-induced damage to the epiphyseal growth plate is a major cause, but other factors can also attribute to impaired growth, e.g. radiation-induced hypothyroidism, hypogonadism or growth hormone deficiency (GHD). Several studies have shown that decrease in height SDS is greater in boys, in younger children and in children with a history of cranial irradiation (CI) or craniospinal irradiation (CSI) [2-6]. Studies on the effect of GH therapy on growth after TBI are limited and almost exclusively deal with children diagnosed as having GHD. We investigated the effect of GH therapy in children with impaired growth after TBI and SCT, irrespective of the diagnosis of GHD.

Materials and methods

In January 1997 we implemented a protocol for the diagnostic evaluation and subsequent treatment of children and adolescents (<16 years of age) with impaired growth after TBI-based conditioning for SCT. The treatment protocol was approved by the Patient Care Committee and Scientific Research Committee of the Department of Paediatrics of the Leiden University Medical Centre, and by the Review Board of the Netherlands' Organisation for Scientific Research (NWO). Informed consent was obtained from all patients involved in this study.

Inclusion criteria

1) TBI-based conditioning and SCT for a haematological malignancy before the age of 14 years *and 2)* at least two years relapse-free survival after SCT *and* 3) impaired growth (decrease in height SDS > 0.5 SD since SCT or height SDS below patient's target height range) *and* 4) absence of other causes of impaired growth (e.g. CI as part of initial treatment, chronic graft-versus-host

disease, pubertal delay, untreated hypothyroidism, prolonged use of corticosteroids).

Diagnostic evaluation

All patients eligible for inclusion were offered evaluation of GH secretion. Priming with sex steroids was used in prepubertal girls ≥ 8 years of age and prepubertal boys ≥ 9 years of age. The peak serum concentration of GH after stimulation with clonidine (0.15 mg/m^2 orally) or arginine (0.5 g/kg i.v.) was used as a measure of stimulated GH secretion. Spontaneous GH secretion was measured by nocturnal GH concentration profiles, using 12 h continuous blood withdrawal (2 ml/h) from 2000 h until 0800 h with a sample interval of 20 minutes. Results were analysed using the Cluster algorithm [7].

Assays and reference values

Serum GH was measured using a time-resolved immunofluorometric assay, specific for the 22-kD form of GH with detection limit 0.01 µg/l, (Delfia hGH IFMA; Wallac, Turku, Finland, calibrated against the international reference preparation 80/505 in which 2.6U=1mg). Plasma levels of IGF-I and IGFBP-3 were determined at the endocrine laboratory of the Wilhelmina's Children's Hospital, Utrecht, the Netherlands. The assays were described previously [8]. Results are expressed as age- and sex-specific SDS.

According to national criteria, peak GH > 20 mU/l after pharmacological stimulation was considered sufficient. Based on nation-wide harmonisation of GH assays, 13.8 mU/l in our assay corresponds to 20 mU/l in the national reference assay. Therefore, GH response was considered insufficient if peak GH was < 13.8 mU/l (5.3 ng/ml).

Criteria for normal spontaneous GH secretion were maximum GH > 5.3 µg/l and mean GH concentration > 1.2 µg/l, based on assay-specific references [9].

Growth hormone deficiency (GHD) was defined as an insufficient peak GH (spontaneous or stimulated) on 2 separate occasions. GH neurosecretory dysfunction (NSD) was defined as sufficient peak GH after pharmacological stimulation but decreased maximum peak GH or mean GH concentration in a 12 hour GH secretion profile.

Treatment and follow-up

After diagnostic evaluation of GH secretion status, all patients were offered treatment with recombinant human GH at a daily dose of 1.33 mg/m^2 body surface area.

Height, Tanner stages of breast or genital development [10] and testicular volume (measured with an orchidometer) were monitored at 3 months' intervals during treatment with GH. Height was measured with a Harpenden stadiometer and expressed as standard deviation scores (SDS) for sex and age [11]. Target height (TH) was calculated according to the formula introduced by Tanner et al [12] with corrections for sex (13 cm) and secular trend (4.5 cm), and TH range was defined as TH SDS +/- 1.3 SDS.

The onset of puberty was defined as the age at which breast development was first recorded in girls (Tanner breast stage ≥ B2) or a testicular volume ≥ 4 mL was reached in boys. If signs of pubertal development (e.g. penile growth and pubic hair development) had occurred in boys while testicular volume had not reached 4mL, onset of puberty was determined on the basis of the combination of progression of Tanner stages and increasing serum levels of testosterone. If puberty was induced, onset of puberty was defined as the start of sex hormone replacement therapy.

After completion of therapy, height was measured at 6 months' intervals until final height (patients were considered to have reached final height if growth was less than 1.0 cm over a period of more than 12 months after the age of 15 years).

Patient population

Between 1997 and 2005, 66 patients met the inclusion criteria (patient characteristics are summarised in table 1). Stimulated and spontaneous GH secretion were evaluated in 29 children, including one patient (nr. 29 in the tables, treated outside the protocol) who did not meet the inclusion criteria (SCT at age 14.3 years and <2 years survival). Twenty-three children started GH therapy (effective mean starting dose 34 µg/kg/day, range 27-39). For two of these patients (nr. 27 and 28), evaluation of GH secretion as well as follow-up of GH therapy took place in another centre, with a different GH assay and a lower starting dose of GH in one (0.67 mg/m^2; corresponding to 18 µg/kg/day).

Table 1. Patient characteristics.
Figures represent either absolute numbers, or medians with ranges in parenthesis.

	All patients (n=66)	GH tested (n=29)	GH treated (n=23)
Male : female	48 : 18	22 : 7	17 : 6
Age HCT	7.7 (1.7-14.3)	7.7 (1.7-14.3)	7.7 (1.7-14.3)
Age GH evaluation	-	12.5 (7.2-15.9)	12.2 (7.2-15.9)
Age last visit	16.6 (8.8-22.4)	16.8 (9.2-19.3)	17.3 (10.8-19.3)
Follow-up since HCT	7.7 (2.0-17.0)	8.1 (2.1-17.0)	9.1 (2.1-17.0)
Follow-up since GH evaluation	-	4.4 (0.0-7.8)	5.1 (0.6-7.8)
Follow-up since start GH	-	-	4.2 (0.5-7.7)
Indication HCT			
ALL 1st	10	6	4
ALL 2nd	27	13	12
AML 1st	11	6	6
AML 2nd	4	1	-
MDS	8	3	1
CML	4	-	-
NHL 2nd	2	-	-
Type of graft			
Allogeneic	60	28	22
Autologous	6	1	1
TBI dose			
5.0 Gy	1	-	-
7.0 Gy	9	5	5
7.5 Gy	37	15	12
8.0 Gy	2	1	-
2 x 6.0 Gy	17	8	6
Testicular Booster	6	4	3

Conditioning for SCT

Conditioning for SCT consisted of TBI and cyclophosphamide (60 mg/kg/day i.v. for 2 consecutive days) in all patients. In addition, 19 patients also received cytarabine (1 g/m^2/day for 2 consecutive days) and 37 patients received etoposide (350 mg/m^2/day for 2 consecutive days). All patients received unfractionated TBI, delivered at a mean instantaneous dose rate of 25 cGy/min. As age is an important determinant of the tolerable total irradiation

dose in children, a TBI regimen with age-dependent total dose was applied (0-2 years: 5.0 Gy, 2-4 years: 7.0 Gy, 4-10 years: 7.5 Gy, >10 years 8.0 Gy). The latter dose was 'increased' in 1989 to 2 single fractions of 6.0 Gy, given on 2 consecutive days (instead of the equivalent 9.0 Gy once, which had too many side effects in adults). Six boys treated for ALL received additional prophylactic testicular irradiation (10 Gy in 4 fractions prior to TBI).

Statistical Analyses

S-PLUS 6 Professional (Insightful corp., Seattle WA, USA) was used for all statistical analyses, with significance level set at 5%. We recently developed sex-specific models that describe the changes in height SDS with time after TBI and SCT, with corrections for the effects of individual puberty and for the effect of puberty in the reference population, using the function 'lme' (linear mixed-effects) in S-PLUS [6]. These models are based on 75 children who had at least 2 years of follow-up after pre-pubertal TBI-based conditioning for SCT for a haematological malignancy. Three of the 23 GH treated children from the present study were not included in this previous study (nrs. 5 and 14 were not pre-pubertal at time of SCT, nr. 29 due to relapse), the others contributed to the initial models until the start of their GH therapy.

For the present analyses, we added the height measurements taken after start of GH therapy of these 20 patients to the dataset (total set 1236 post-TBI height measurements of 75 patients) and subtracted the *individual* growth profiles (obtained from the model) from the individual height SDS after start of GH therapy. The resulting values describe the difference between *individually* predicted growth (i.e. without GH therapy) and actual growth after start of GH therapy. This estimated effect of GH therapy was then analysed by adding a variable 'time since start GH therapy' to the model with a non-linear (logit) effect. To prevent possible selection bias by exclusion of patients who discontinued therapy due to a poor response, analyses of the effect of treatment were done on an intention-to-treat basis. Therefore all data obtained after start of therapy was included in the analyses, even if GH therapy was discontinued by that time.

Table 2. Results of the evaluation of GH secretion status in 29 patients with impaired growth after HCT. Patients 27 and 28 were tested in another centre; patient 29 was treated outside the protocol (< 2 years disease free survival at time of evaluation)

				Spontaneous GH secretion		Spontaneous GH secretion					
Patient	sex	Age GH Evaluation	Tanner stage	Mean GH (µg/l)	Peak GH (µg/l)	Peak 1 GH (µg/l)	Peak 2 GH (µg/l)	IGF-1 (SDS)	IGFBP-3 (SDS)	Diagnosis	GHRx
1	M	13.3	1	0.4	1.9	3.6	4.1	-1.8	-1.8	GHD	yes
2	M	14.3	2	0.5	3.8	7.8		-1.3	0.5	GHNSD	yes
3	M	8.8	1	0.6	3.1	4.8		-0.6	1.0	GHNSD	no
4	M	13.3	2	0.9	3.7	5.0	5.5	-1.9	-1.1	GHNSD	yes
5	M	14.2	2	-	-	0.7	4.5	-0.5	-1.7	GHD	yes
6	M	14.1	3	1.0	7.7	12.6	21.7	1.8	0.7	GHNSD	no
7	M	11.1	1	1.3	4.4	8.4		-1.5	0.8	normal	yes
8	M	7.2	1	1.4	8.0	17.8		-1.0	-2.0	normal	yes
9	F	11.9	2	1.5	7.3	4.2		0.2	1.0	Normal	no
10	M	9.7	1	1.7	9.9	11.7		-0.7	0.8	normal	yes
11	F	12.5	1	1.9	9.2	5.9	8.1	-1.7	0.7	normal	yes
12	M	12.8	1	2.0	12.2	12.4		-0.9	0.0	normal	yes
13	M	13.2	3	2.0	10.6	8.3		0.4	1.4	normal	no
14	F	15.1	3	2.0	6.1	15.5		-2.3	-0.9	normal	yes
15	M	7.4	1	2.0	11.2	12.4		0.6	-0.4	normal	yes
16	F	7.6	1	2.1	11.0	7.0	5.1	1.3	2.4	normal	yes
17	M	12.6	2	2.1	13.3	19.3	11.5	-0.8	1.1	normal	yes
18	F	14.1	4	2.3	9.2	3.8	15.9	-1.0	-0.3	normal	yes
19	M	12.1	2	2.4	15.0	8.0		-0.4	1.0	normal	yes
20	M	11.3	1	2.4	13.3	9.4		0.5	0.9	normal	yes
21	M	12.2	2	2.5	13.3	9.6	2.2	-0.5	0.9	normal	yes
22	F	12.9	3	2.7	23.3	12.2		0.2	1.2	normal	yes
23	F	10.3	1	2.9	8.7	18.5	10.2	-1.8	-0.1	normal	yes
24	M	11.2	2	2.9	10.2	7.9		0.4	1.2	normal	yes
25	M	15.2	4	3.6	27.3	11.4		-1.3	0.4	normal	no
26	M	12.9	3	6.2	21.7	24.3		0.0	2.0	normal	no
27	M	10.7	1	4.3	-	25.8	7.7	-2.1	-0.8	normal	yes
28	M	9.9	1	1.6	-	24.	0.8	-2.7	-1.0	GHNSD	yes
29	M	15.9	2	0.9	5.8	7.1	4.6	-3.3	1.2	GHNSD	yes
		Reference median (range):		2.2 (1.2 – 7.9)	10.5 (5.1- 36.5)						

Results

Growth hormone secretion

Results of the analyses of GH secretion are represented in table 2. GH secretion was impaired in 7 of the 27 children tested in our clinic, including the patient treated outside the protocol. Two patients had classical GHD, five had NSD. Of the two patients tested in another centre (27 and 28), one had a decreased integrated mean spontaneous GH secretion using assay-specific reference values for that centre [13].

Effect of GH therapy

Table 3 summarises height SDS at different time points in the children receiving GH therapy. The median duration of GH therapy was 3.2 years (range 0.1-7.3 years).

Table 3. Target height SDS and height SDS at different time-points in the 23 patients treated with GH. ***** Adult height: Final height expressed as SDS for age 21 years.

Patient	Sex	Duration GH Rx (years)	follow up since start GH (years)	Target height (SDS)	Height at SCT (SDS)	Height at start GH (SDS)	Delta height after start GH (SDS)	Adult height* (SDS)
1	m	1.5	1.5	-1.7	-2.3	-3.5	0.1	
2	m	1.9	4.0	1.1	1.2	0.1	0.0	-0.1
4	m	3.3	4.1	1.6	-0.3	-0.9	0.5	
5	m	2.1	2.6	-2.2	-0.6	-2.6	0.0	
7	m	4.0	4.0	-0.3	-0.5	-1.6	-0.3	
8	m	7.2	7.2	0.0	0.4	-0.4	0.6	
10	m	6.4	6.4	-0.7	-2.7	-2.4	0.2	
11	f	3.6	5.4	0.6	-0.2	-1.0	1.0	-0.1
12	m	2.6	4.7	-0.2	-1.0	-1.8	-0.6	-2.7
14	f	0.1	2.5	-0.3	-1.5	-2.0	0.1	-2.0
15	m	3.1	3.1	0.6	-1.2	-1.7	1.1	
16	f	5.7	6.3	1.0	-2.2	-2.0	0.4	
17	m	3.1	6.4	1.1	-0.9	-1.2	-0.8	-2.4
18	f	1.6	3.6	0.0	-0.7	-1.5	0.2	-1.4
19	m	3.0	5.8	1.6	1.2	0.2	0.4	0.4
20	m	2.3	2.3	1.1	-1.1	-1.5	0.1	
21	m	3.3	3.3	-0.1	-1.5	-1.4	0.2	
22	f	0.7	4.2	0.7	-0.7	-1.7	0.2	-1.6
23	f	4.9	7.7	0.2	-1.0	-3.4	0.4	-3.2
24	m	4.3	5.3	1.2	-0.5	-0.3	-0.4	-1.7
27	m	3.8	4.2	-1.4	-3.0	-3.5	-0.2	-4.0
28	m	5.3	5.3	0.4	0.6	-0.8	0.3	
29	m	0.7	0.7	-0.8	-1.1	-1.6	0.3	

Figure 1 represents the relative changes in height SDS in the 23 children treated with GH compared to height SDS at start of GH therapy. In the first year of therapy, there was a mean change in height of +0.35 SDS/year. Two patients discontinued therapy and one patient (nr 29, treated outside the protocol) died 27 months after SCT after relapse of his initial disease. In 17 of the remaining 20 children, there was an increase in height SDS in the first year of therapy, whereas in 3 there was a slight decrease (<0.05 SDS/year).

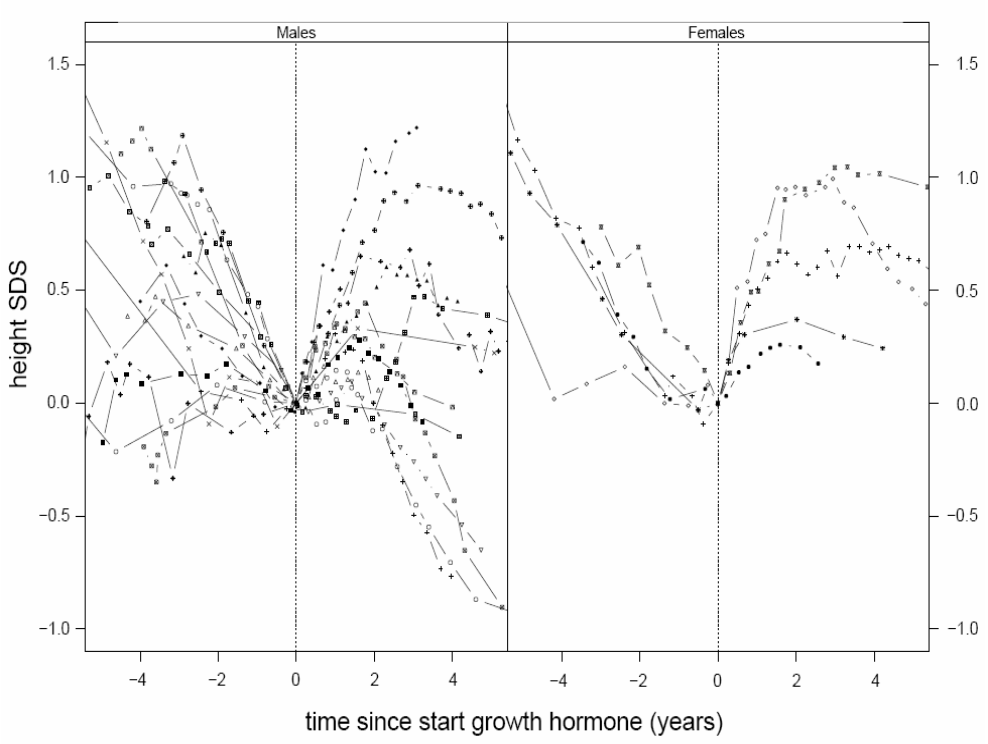

Figure 1. Changes in height SDS after start of GH therapy in the 23 GH-treated children. The x-axis represents time since start of GH therapy in years, the y-axis height SDS compared with height SDS at start of GH therapy. Left panel: males, right panel females.

The estimated net effect of GH therapy in the 20 patients who received SCT before the onset of puberty is represented in figure 2. The estimated net effect after 5 years of therapy is 1.14 (CI 0.88-1.41) SDS, with no significant difference between sexes (p=0.565).

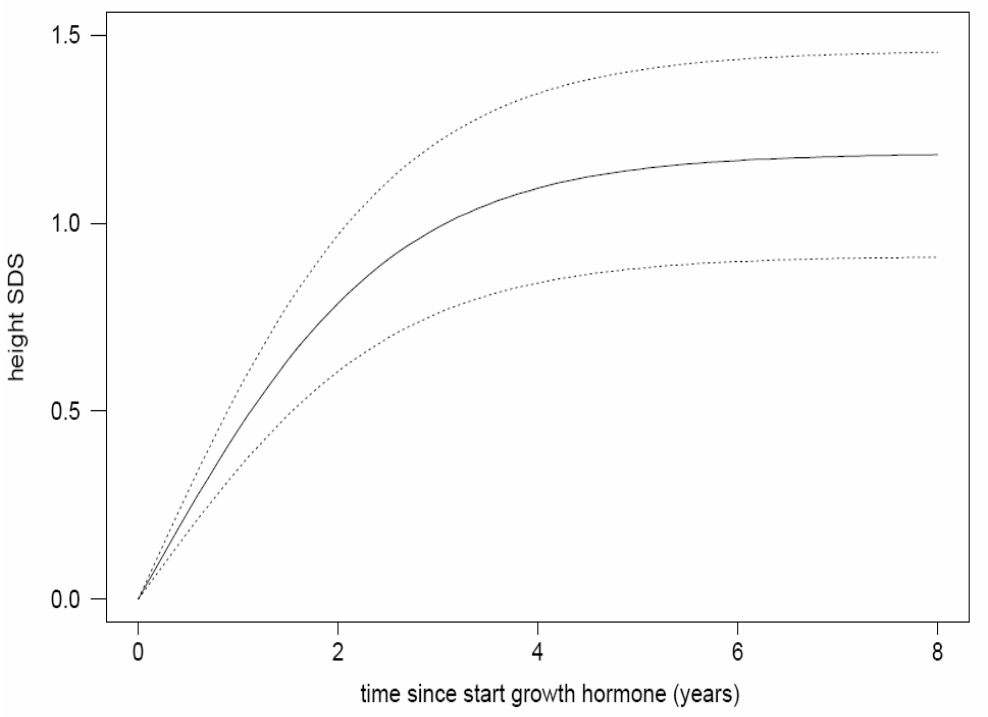

Figure 2. Result of mixed-effect modelling: estimated net effect of GH therapy on development of height SDS in the 20 patients receiving HCT before onset of puberty. Dashed lines represent 95% CI.

Predictive value of parameters of GH secretion

To evaluate the role of GH secreting capacity in impaired growth after SCT, we added different parameters for GH secretion (integrated mean GH concentration, IGF-1 and IGFBP-3) to the mixed-effect model. None of the parameters had a significant effect on either loss of height SDS after SCT or on the increase in height SDS after initiation of GH therapy.

Adverse effects, relapses and secondary tumours

Within 2 weeks after start of therapy, patient nr. 14 repeatedly had urticaria and angioedema after injecting GH and decided to stop GH therapy. Due to increasing aversion of injections, patient nr. 22 decided to stop GH therapy after eight months.

In the 43 untreated patients compensated hypothyroidism was diagnosed in 12 (28%) and autoimmune hypothyroidism in 1 (2%). In the 23 GH treated patients, compensated hypothyroidism was diagnosed in 8 (35%) before start of therapy, and in an additional 8 (35%) after start of therapy. Thyroxine suppletion was initiated in all patients with compensated hypothyroidism.
Five boys and one girl developed exostoses during GH therapy, another boy experienced growth of pre-existing exostoses during GH therapy. In the 43 untreated children, two boy developed exostoses. There was 1 relapse among the 23 GH treated patients (nr 29; treated outside the protocol relapsed 9 month after start of GH therapy), compared to 6 relapses in the 43 untreated children. In the untreated group, one patient developed a schwannoma 7.3 years after TBI. In the GH treated group 2 patients developed a secondary malignancy. Patient 28 developed an osteosarcoma 5.3 years after start of GH therapy (12.7 years after SCT). Patient nr. 22 (treated with GH for only 8 months) developed papillary thyroid carcinoma 3.5 years after start of GH therapy (9.7 years after SCT).

Discussion

This study reports a positive effect of GH therapy on height SDS in children with impaired growth after TBI-based conditioning for SCT, even in the absence of GHD. The calculated net effect (1.1 SD after 5 years of GH therapy) did not correlate to GH secretion status. A potential limitation of our study is the use of single fraction TBI, as most SCT centres use fractionated TBI. As final height results from our centre are comparable to those reported by centres using fractionated TBI, we believe this to be a minor limitation.
GHD was diagnosed in 8 of the 66 patients (12%) monitored since 1997. There is a wide variation in the reported incidence of GHD after TBI-based conditioning for SCT, with incidences from 0 to 84% [3-5;14-27]. Factors contributing to these differences in incidence of GHD are: population size, duration of follow-up, TBI characteristics (dose, dose rate, fractionation), indications for GH testing and selection bias (e.g. exclusion of children with a history of CI or exclusion of children not tested for GHD). In addition, the criteria used for the diagnosis of GHD largely influence the incidence of GHD. If we had used ‘standard’ criteria for GHD (e.g. mean nocturnal GH concentration < 2.5 µg/l or peak GH < 10 µg/l on two occasions, 23 of our 29 patients tested

(79%) would have been diagnosed as having GHD. The vast majority of the studies on GHD after TBI do not mention the GH assay used, nor do they mention the use of assay specific references. We believe that the relatively low incidence of GHD in the present study can be attributed to the absence of children with additional CI as well as to the use of assay-specific criteria for the diagnosis of GHD.

Most patients included in the present study showed a decrease in height SDS between TBI and start of GH therapy (figure 1). A continuing decrease in height SDS could be expected based on previous reports in the literature as well as our model for growth after TBI. However, in most of the patients treated with GH, height SDS at last visit (mean follow-up of 4.4 years) was comparable to height SDS at start of therapy (table 3).

Data on the effect of GH therapy on height after TBI-based conditioning for SCT are limited, with only 3 studies reporting final height [2;4;5]. The first study by Cohen et al. is a questionnaire-based study on final height after pre-pubertal SCT in the European EBMT centres, showing no significant difference in final height between GH treated an non-GH treated patients [2]. Due to the set-up of the study, no information was available on the indications for GH therapy, the GH doses used or the changes in height SDS after start of GH therapy. Therefore, no valid conclusions can be drawn from this study regarding effect of GH therapy. The second study by Frisk et al. reports final heights of 11 patients treated with GH after TBI and autologous SCT for ALL (6 had received additional CI and one CSI). In that study, 6/11 GH treated patients were considered to have GHD based on maximum GH peak in 12- or 24-hour spontaneous GH secretion profiles. The GH assay (and probably also the reference standard) was the same as in the present study, but the cut-off for the diagnosis of GHD was much higher (maximum GH < 10 µg/l vs 5.3 µg/l in our study). According to multiple regression analyses, the effect of GH therapy on height SDS was 0.18 SDS for each year of GH therapy. The third study by Sanders et al. reports the effect of GH therapy on 'final height' (height at the age of 16 years) of 90 TBI patients (32 CI) diagnosed with GHD, 42 of whom (21 CI) were treated with GH (20 - 30 µg/kg/day). According to multiple regression analysis, GH treatment resulted in 0.86 SD increase in final height in the 35 children with SCT before the age of 10 years, whereas no significant effect of GH therapy was found in the 7 children receiving SCT after the age of 10 years.

The estimated net effect of GH therapy on height SDS in our study (1.1 SDS after 5 years) is comparable to that reported by Sanders et al. in children receiving SCT before the age of 10 years (0.9 SD), as well as to that reported by Frisk et al. (0.18 SD/year). Major differences with these previous studies are that the majority of the patients in the present study were not considered GH deficient (although diagnostic criteria differ between the studies [28]), and that the estimation of the effect in the present study is based on mixed-effect modelling, a robust statistical method that allowed us to predict individual height SDS curves and compare those to actual height SDS after start of GH therapy without the risk of significant bias and without the need for final height in all patients.

We also looked at the influence of GH secretion status on either decrease in height SDS until testing and on the effect of GH treatment. The results of GH secretion status did not correlate with decrease in height SDS before GH therapy or with response to GH therapy. This suggests that impaired growth after SCT is mainly caused by radiation damage to the epiphyseal growth plate (and thus end-organ sensitivity) and far less by decreased GH secretion.

The incidence of (sub-clinical) hypothyroidism was more than twice as high in the GH treated patients compared to the non-treated patients. The reported incidence of radiation induced hypothyroidism after TBI in children is 15-50% and seems to be higher after unfractionated TBI [29]. In GH deficient children, GH replacement therapy does not induce primary hypothyroidism. It either reveals previously unrecognised cases of central hypothyroidism or induces hypothyroxinaemia by increased conversion of thyroxine to tri-iodothyronine [30-32]. None of our patients had central hypothyroidism, however, and the majority of cases were not GH deficient. The higher incidence of compensated hypothyroidism in GH treated survivors of TBI is in line with the results of Sanders et al. [5]. A possible explanation for this higher incidence of hypothyroidism in GH treated children could be that in GH treated patients thyroid function was monitored more closely, or that increased growth increased the demand for thyroxine.

The risk of secondary cancers is increased in all survivors of TBI-based conditioning for SCT during childhood and cumulative incidence increases with time after SCT and in case of younger age at SCT [33]. Although surveillance studies do not suggest an increased risk of disease recurrence in survivors of childhood cancer by GH therapy, potential oncogenic effects of GH remain a

concern [34;35]. In supra-physiological doses both GH and IGF-I stimulate proliferation and differentiation of both normal and leukaemic cultured human lymphocytes [36;37], more physiological concentrations of GH (i.e. less than 50 ng/mL), however, do not have an effect on colony formation, nor on the number of colonies or DNA-synthesis in cultured leukaemic cells. In the present study, two of the 23 GH treated patients developed a secondary tumour (one thyroid carcinoma and one osteosarcoma), compared to 1 out of the 43 untreated patients.

Relapse of leukaemia occurred less frequently in the GH treated patients (1/23) compared to untreated patients (6/43; including one patient who considered starting GH therapy when his leukaemia relapsed almost 5 years after SCT).

Nine of our 66 patients (13.6%, only one female) developed exostoses (believed to be osteochondromas). Exostoses were more frequent in the GH treated group (7/23, 30%) compared to the non-treated group (2/43, 4.7%). Osteochondromas develop in 9-23% of children with TBI-based conditioning for SCT [38-40]. A role for GH therapy in promoting their development is suggested, but malignant degeneration is believed to be rare [5;38-40].

In conclusion, our study shows that recombinant human GH (± 33 µg/kg/day) has a positive effect on height SDS after SCT, even in the absence of GHD. We could not establish a relation between decrease in GH secretion and either impaired growth after SCT or response to GH therapy (probably due to interference of growth plate damage). We therefore believe that evaluation of GH secretion has limited value in predicting the response to GH therapy, and treatment could be considered in every patient with severe growth impairment after TBI, even in the absence of GHD. Patients should be informed about the increased risk of secondary tumours after SCT and the concerns of oncogenic potential of GH therapy, as well as about the possibility of increased risk of osteochondromas.

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

Bone Marrow Transplantation 2004;33:1049-1056

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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).

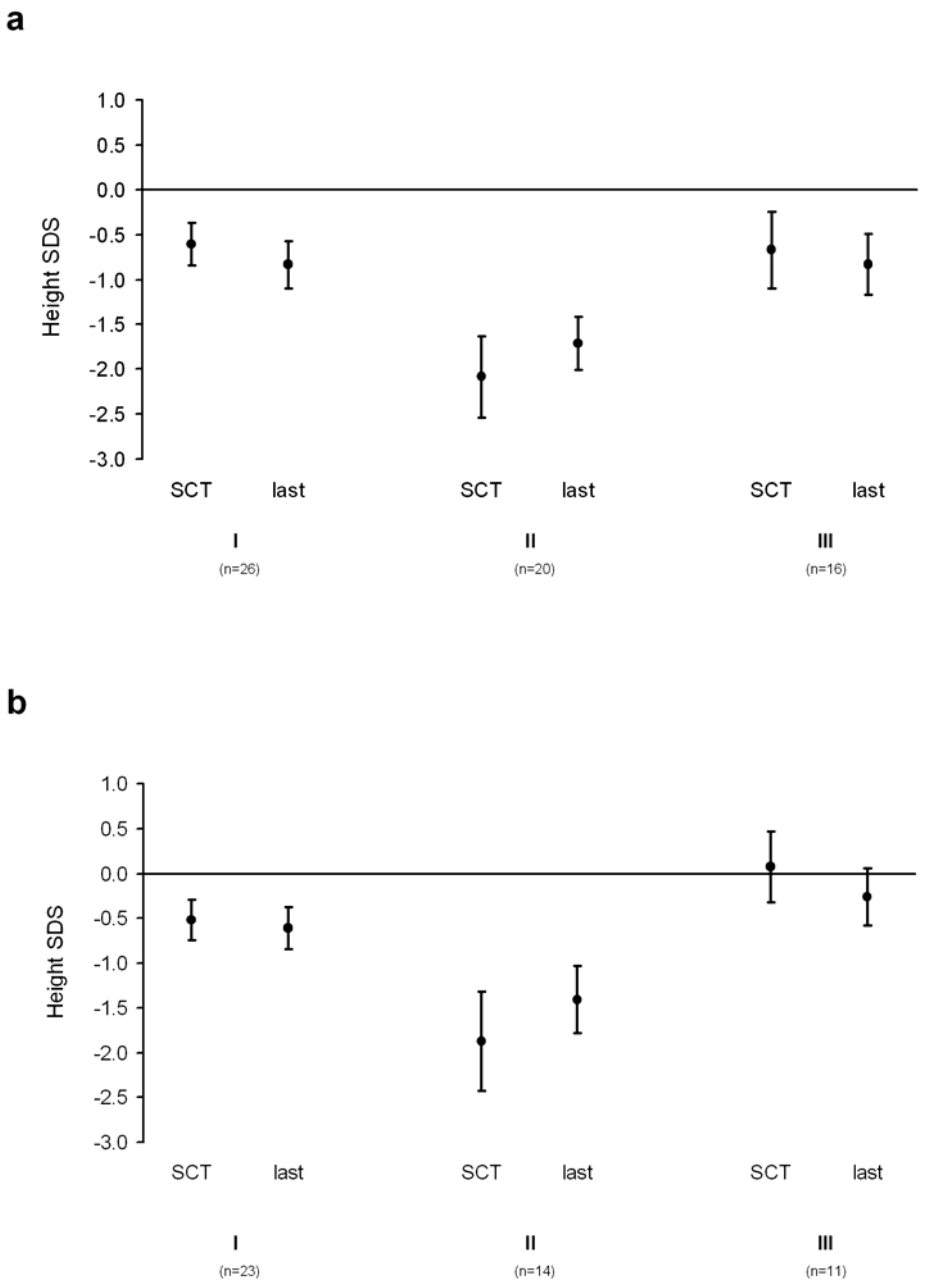

Figure 1. Changes in height SDS after SCT. The mean height SDS in the three subgroups at SCT and last visit. (a) Represents all patients (n=62). (b) Represents patients without growth-limiting disorders (n=48). Error bars represent standard error of the mean.

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	Indication SCT	Gonadotoxic treatment prior to conditioning	Bu/Cy (mg/kg)	Gonadal 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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QUALITY OF LIFE IN ADULTS FOLLOWING BONE MARROW TRANSPLANTATION DURING CHILDHOOD

Bone Marrow Transplantation 2004;33:329-336

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Abstract

To Quality of life (QOL) was assessed in 22 young adults, 14 years -on average- after having received bone marrow transplantation (BMT) during childhood at the Leiden University Medical Centre. All were disease-free and >16 years when interviewed. The sickness impact profile and the Medical Outcome Study 36-item Short Form Health Survey were used as generic questionnaires in the assessment of QOL. The Functional Assessment of Cancer Therapy-Bone Marrow Transplant Scale (FACT-BMT) was used as a disease-specific measure of QOL. Coping was assessed by means of the Utrecht coping list. BMT-related variables were obtained from medical files. Of the generic QOL measures, most results fell within the normal range of functioning, although some illness-related impairment was reported on subscales for general and work-related functioning. Compared to a reference sample of patients who had received BMT as adults, patients involved in this study scored significantly higher on the 'emotional well-being' subscale of the FACT-BMT, indicating significantly better emotional functioning. The age at BMT and total body irradiation (TBI) were not related to patients' QOL. We can conclude that at long term, having received BMT during childhood does not negatively affect the QOL of patients.

Introduction

Bone marrow transplantation (BMT) has become a standard treatment option for many children with congenital or malignant disorders of the haematological system [1]. It is an intensive procedure associated with lengthy hospitalisation (for some time in protective isolation), and risk of severe pre-treatment and treatment-related morbidity [2;3]. As the number of survivors of BMT increases, the long-term effects of this treatment procedure on the quality of life (QOL) of patients and their family members are becoming more important.

QOL has been defined as 'the functional effect of an illness and its consequent therapy upon a patient, as perceived by the patient'. [4] QOL research has long been plagued by a lack of consensus on the definition of the core concepts and by a lack of standardisation of assessment tools [5;6]. QOL measures include generic and disease-specific ones. While generic measures of QOL can be used across different patient populations, the disease-specific ones include aspects of health (symptoms, impairments, and disabilities) that are relevant to patients with a particular disease. The choice of measure to use in an investigation into QOL is a difficult one to make [7]. Inherent to the assessment of QOL are also a number of methodological considerations, including the phenomenon of 'response shift' (a change in the meaning of an individual's self-reported QOL), that can affect the validity of the measures used [8-11]. However, despite the ongoing debate on the meaning of QOL, its assessment, and its usefulness in health care [5-16], most would argue that assessing the QOL when investigating the impact of a clinical intervention provides valuable information on patient outcome [17].

Studies conducted on the QOL of *adult* BMT recipients have yielded contradictory results. While some studies indicate that the QOL of adult recipients is relatively unaffected, at long term, others report a wide variety of problems, including low energy levels and sleep difficulties, low self-esteem, sexual difficulties, psychological distress, and impaired social relationships [18-25]. Two recent reviews highlight these contrasting findings and attribute them, in part, to differences in methodology [26;27].

Notwithstanding the differences in methodology, the reviews point to some interesting research findings. Firstly, age at BMT seems to play an important role in the QOL of adult BMT recipients, younger BMT recipients doing better

following BMT [26]. Secondly, TBI dose seems to be related to poorer sexual, cognitive, and physical functioning [26]. Thirdly, the time post BMT has been found to be unrelated to psychosocial status, functional QOL, and affective status [26]. Fourthly, fatigue, psychological distress, and sexual dysfunction are frequently reported following BMT [27].

The long-term effects of having received BMT during *childhood* or *adolescence* have hardly been studied. A recent prospective longitudinal study on children who received haematopoietic stem cell transplantation (HSCT) indicated that both at 1 year and 2 years post HSCT, there was a low prevalence of behavioural and social problems in children [28]. These findings support the results of a recent cross-sectional retrospective study on paediatric patients who received a BMT 1–13 years earlier. Most patients (75%) reported no physical or psychological impairment [29]. Another cross-sectional study revealed that young adults who underwent BMT during childhood reported fewer problems when compared with their healthy peers with respect to interpersonal relationships, sleep, depression, and leisure possibilities, 3-9 years after BMT [30]. On the other hand, they reported more problems with regard to their physical appearance, and their studies and work possibilities [30]. A fourth cross-sectional retrospective study revealed that patients who underwent stem cell transplantation (SCT) between the ages of 5 and 18 years were at a risk of developing long-term emotional or social problems, 2-13 years later [31]. In turn, a fifth study assessing the behavioural adjustment, QOL, and adaptive functioning of children and adolescents, pre- and 6 months post BMT, revealed an improvement in overall QOL at 6 months post BMT, and no symptoms of serious psychological maladjustment at either pre- or 6 months post BMT (as rated by their mothers) [32].

The aim of this study is to assess the long-term effects of having undergone BMT in childhood on QOL in young adulthood. In addition to assessing the health-related QOL of young BMT recipients, we included demographic and BMT-related variables (e.g. age, gender, age at BMT, intelligence quotient (IQ)) into our investigations. Recent studies have indicated that these variables may be associated with the QOL of BMT recipients [26;33;34]. For example, younger BMT recipients have been found to overcome BMT-related toxicities more readily than adult BMT recipients, females have been found to be at a greater risk of developing sexual problems post BMT, TBI dose has been reported to be associated with BMT patients' cognitive functioning, and higher

intellectual ability has been linked to better coping with childhood cancer and its treatment [26;33;34]. Furthermore, research has indicated that patients' QOL is not determined exclusively by disease-related factors. Psychological concomitants of an illness, such as the way in which patients cope with it, have been found to play a crucial role [35-39]. Thus, in addition to demographic and BMT-related variables, we aimed at investigating the role of coping in the QOL of young adults who underwent BMT during childhood.

Patients and methods

Patients and procedure

The assessment of QOL was part of a larger investigation into the long-term consequences of BMT during childhood. The study was approved by the Medical Ethics Committee of the Leiden University Medical Centre (LUMC). Individuals who were invited to participate in these investigations were recruited from the total number of patients who had received allogeneic BMT at the department of Paediatrics of the LUMC between 1968 and January 1993. They were selected on the basis of five criteria: 1. having received a (first) BMT at least 5 years prior to the study; 2. having been below the age of 18 years when receiving BMT; 3. having received BMT on the basis of the indication of haematological malignancy or severe aplastic anaemia; 4. being 16 years or older when participating in the current study; and 5. the disease being in complete remission when participating.

A total of 61 patients met the selection criteria. In all, 12 patients could not be contacted, because they had moved house without leaving a forwarding address, or because they had moved to another country. Thus, a total of 49 patients were invited to participate, of whom 22 participated. Signed informed consent was obtained from all participants. The characteristics of both participants and non-participants are summarised in table 1.

The health status of participants was compared to non-participants' by chart review. Health status did not differ between the two groups: 7/22 participants suffered from severe transplant-related morbidity (e.g. severe chronic graft-versus-host disease or secondary neoplasms) compared to 5/27 non-participants. The reasons for not participating were: the time-consuming nature of the study (n=8, 30%), patients attending another hospital for their regular

check-ups (n=7, 26%), reasons not given (n=5, 19%), patients not considering themselves as being ill (n=4, 15%), and the study being regarded as too physically and emotionally taxing (n=3, 11%).

Table 1 Sample characteristics

	Participants		Non-participants	
	N (%)	Mean (s.d.; median; range)	N (%)	Mean (s.d.; median; range)
Sex				
- Female	12 (54.5%)		12 (44.4%)	
- Male	10 (45.5%)		15 (55.6%)	
Age (years)		25 (5; 24; 18–32)		22 (5; 21; 18–36)
Age at BMT (years)		11 (4; 11; 1–16)		11 (3; 12; 5–17)
Time since BMT (years)		14 (4; 14; 6–21)		11 (4; 11; 6–26)
Indication of BMT				
- Severe aplastic anaemia	4 (18.2%)		6 (22.2%)	
- Acute myeloid leukaemia	9 (40.9%)		11 (40.7%)	
- Acute lymphoblastic leukaemia	5 (22.7%)		7 (25.9%)	
- Chronic myeloid leukaemia	1 (4.5%)		0 (0%)	
- Myelodysplastic syndrome	2 (9.1%)		2 (7.4%)	
- Non-Hodgkin's lymphoma	1 (4.5%)		1 (3.7%)	
TBI dose				
- No TBI	3 (13.6%)		2 (7.4%)	
- 4–5 Gy	2 (9.1%)		4 (14.8%)	
- 7–8 Gy	13 (59.1%)		13 (48.1%)	
- 2 x 6Gy	4 (18.2%)		8 (29.6%)	
IQ		110 (16; 81–131)		

Patients who agreed to participate were invited to attend the LUMC to undergo a series of tests and complete a number of questionnaires. Pulmonary function, endocrinological variables, ophthalmological variables, and QOL were assessed. In this paper, we will report the findings on the patients' QOL.

Results of other investigations (e.g. lung function and renal function) will be reported elsewhere.

Measures Demographic and BMT-related variables

Patients were asked to report their age and sex and, as a part of the larger study, patients' IQ was assessed by means of the Wechsler adult intelligence scale-revised (WAIS-R) [40]. Information on patients' disease characteristics (e.g. indication for BMT, age at BMT, use of TBI) was obtained from their medical files.

QOL measures

As mentioned earlier, choosing the appropriate measure to investigate the QOL of a given patient sample is difficult [7]. For the purpose of the study described here, we chose to assess BMT patients' QOL by means of two widely known generic measures of QOL, with proven reliability and validity, in addition to using a recently developed disease-specific measure of QOL, which has not yet been used in a paediatric sample. By this means, we intended to benefit from advantages offered by both generic measures (e.g. availability of data for comparison across different patient populations and from large community samples) and disease-specific ones (greater sensitivity to changes in QOL caused by factors associated with a specific health problem) [41].

Thus, we used two generic QOL measures: the sickness impact profile (SIP) and the Medical Outcome Study 36- item Short Form Health Survey (MOS SF-36) [42;43]. The SIP and the SF-36 have proven to be reliable and valid instruments, and have been used in a wide variety of patient populations, including patients with cancer [44-48]. The SIP focuses on the impact of an illness as reported by the patient, and contains 136 items in 12 categories, from which three additional aggregate scores can be calculated ('physical dimension', 'psychosocial dimension', and 'total score'). SIP scores are presented as percentages of maximal dysfunction ranging from 0 to 100, higher scores indicating higher level of dysfunction [42]. SIP scores lower than 6 indicate no impairment, scores between 6 and 10 are indicative of mild impairment, scores between 15 and 20 indicate moderate to severe impairment, and scores above 20 are indicative of severe illness-related impairment [49]. The MOS SF-36 comprises 36 items in eight functional dimensions: 'physical functioning', 'role functioning - physical', 'bodily pain', 'general health', 'vitality', 'social

functioning', 'role functioning - emotional', and 'mental health'. The raw scores are transformed in order to obtain a 0–100 scale, higher scores indicating better functioning [43].

To assess the disease-specific QOL of BMT patients, we used the 4th version of the Functional Assessment of Cancer Therapy-Bone Marrow Transplant Scale (FACTBMT) [50]. It is a valid and reliable measure of QOL in BMT patients [50;51]. The FACT-BMT consists of the 35-item Functional Assessment of Cancer Therapy (FACT-G) and a 12-item Bone Marrow Transplantation Subscale (BMTS). The latter assesses specific BMT-related issues. The FACT-BMT assesses the effects of cancer therapy on patients in four areas: physical well-being, social/family well-being, emotional well-being, and functional well-being. A higher score indicates better QOL.

In addition, we assessed the strategies patients used to cope with problems in daily life by means of the Utrecht Coping List (UCL) [52;53]. The UCL is a reliable and valid Dutch questionnaire consisting of 49 items that are categorised into seven scales: 'seeking distraction', 'expressing emotions', 'seeking social support', 'avoiding', 'fostering reassuring thoughts', 'passive coping', and 'active coping'. Patients were asked to rate how often they adopted certain coping behaviours on a four-point scale, ranging from 'seldom/never' to 'very often'. Higher scores indicate a more frequent use of a given coping strategy.

Statistical analyses

Firstly, to investigate the impact of having received BMT in the past on patients' QOL, we compared patients' scores on the SIP, MOS SF-36, FACTBMT, and UCL, to the scores of reference samples, by means of t-tests. Secondly, we conducted simple Pearson correlation analyses between patients' demographic and BMT-related variables, and patients' scores on the SIP, MOS SF-36, FACT-BMT, and UCL.

Results

Sample characteristics

Table 1 describes the characteristics of the sample involved in the current study. The sample consisted of 12 females (54.5%) and 10 males (45.5%) with a mean age of 25 years (s.d.=5; range=18–31 years) and a mean IQ score at

the time of assessment of QOL of 110 (s.d.=16). The mean age at which the patients had received BMT was 11 years (s.d.=4). All patients received allogeneic transplants. Conditioning for BMT consisted of cyclophosphamide (60 mg/kg once daily for 2 consecutive days), in most patients combined with TBI. TBI was delivered by a linear accelerator with energies of either 5.0 or 6.0MV, and at a midline instantaneous dose rate of approximately 23 cGy/min. Patients receiving BMT for SAA received either no TBI or 4 Gy single-fraction TBI. One patient, receiving BMT for MDS in the first year of life, received 5 Gy single-fraction TBI. The remaining patients received either a single fraction of 7.5-8.0 or 12 Gy in two fractions.

Generic QOL

Compared to reference data from randomly selected Dutch individuals (n=192), between the ages of 18 and 30 years, who were interviewed as part of a validation study (n=594) of the Dutch version of the SIP [54], the scores of BMT patients did not differ significantly from those of reference individuals, except on the aggregate 'total score' of the SIP, with BMT patients functioning worse than reference individuals. Furthermore, the scores of BMT patients on the 'work' subscale of the SIP fell into the 'severe illness-related impairment' range of SIP scores (7/22 BMT patients scored 30 or higher on the 'work' subscale; table 2). Of the BMT patients involved in this study, three were unemployed, of whom one was unable to work because of a physical handicap. The remaining participants were either employed or were fulltime students.

When compared to healthy reference Dutch individuals, ages 25-34 years (n=221), who were involved in a study on the validation of the Dutch version of the MOS SF-36 [55], the scores of BMT patients again did not differ significantly from those of reference individuals, except where general health was concerned. BMT patients scored significantly lower on the 'general health' subscale (18/22 BMT patients scored 75 or lower on this subscale), indicating significantly worse functioning (table 3).

Table 2. Mean scores (and standard deviations) of BMT patients (n=22) compared to the scores of a reference sample of randomly selected Dutch individuals (n=192) [54] on the sickness impact profile.

SIP	BMT patients Mean (s.d.)	Reference individuals Mean (s.d.)	t
Sleep and rest	4.4(7.2)	2.8 (5.5)	1.0
Eating	0.9(2.1)	0.9 (2.8)	—
Work	20.4(29.7)	6.7 (19.4)	2.1
Home management	5.6(10.3)	1.2 (5.5)	2.0
Recreation and pastimes	5.4(8.9)	4.2 (9.7)	0.6
Ambulation	2.3(5.9)	0.5 (2.8)	1.4
Mobility	1.2(3.3)	0.5 (2.8)	1.0
Body care and movement	1.7(2.5)	0.5 (1.4)	2.2
Social interaction	4.2(5.1)	2.1 (5.5)	1.8
Alertness behaviour	4.8(10)	2.7 (6.9)	1.0
Emotional behaviour	5.6(7.9)	3.0 (6.9)	1.5
Communication	0.4(1.9)	0.6 (2.8)	-0.4
Physical dimension	1.6(2.6)	0.5 (1.4)	2.0
Psychosocial dimension	3.8(4.4)	2.1 (4.2)	1.7
Total score	3.9(3.9)	1.7 (2.8)	2.6**

** P<0.01.

Table 3 Mean scores of BMT patients (n=21) and of a reference sample of randomly selected Dutch individuals (n=221) [55] on the Medical Outcome Study Short Form Health Survey

MOS SF-36	BMT patients Mean (s.d.)	Reference individuals Mean (s.d.)	t
Physical functioning	80.4(24.4)	89.5 (17.8)	-1.7
Role functioning-physical	73.8(33.0)	82.5 (32.4)	-1.2
Bodily pain	83.4(19.0)	84.1 (23.9)	-0.2
General health	62.9(16.1)	77.5 (19.7)	-3.9***
Vitality	61.4(17.4)	69.1 (19.0)	-1.9
Social functioning	79.2(22.5)	90.7 (16.5)	-2.3
Role functioning-emotional	90.0(21.9)	86.8 (29.6)	0.6
Mental health	75.6(13.3)	78.8 (17.5)	-1.0

*** P<0.001.

Disease-specific QOL

Compared to a sample of 56 adults who received a BMT in adulthood participating in a prior study by Kopp et al. (mean age=34.01, s.d.=9.73; mean time from BMT until study (in months)=44.82, s.d.=38.56) [51], patients involved in the current study scored significantly higher on the 'emotional well-being' subscale, indicating significantly better emotional functioning (table 4).

We were unable to compare our results on the FACT Total (Fact-G) and the BMTS to the findings of Kopp et al. [51], because we used the fourth version of the FACT-BMT, as opposed to Kopp and colleagues who used the third version of this assessment scale. The fourth version of the FACT-BMT includes an additional subscale (relation with doctor), and incorporates 12 items in the calculation of the BMTS, whereas in the third version only 10 items are scored.

Table 4 Scores of BMT patients (n=21) on the Functional Assessment of Cancer Therapy-Bone Marrow Transplant Scale (FACT-BMT) compared to those of a reference sample of adult BMT recipients (n=56) [51] by means of t-tests.

FACT-BMT	BMT patients[a] Mean (s.d.)	Reference individuals[b] Mean (s.d.)	t
Physical well-being (0–28)	23.9 (3.8)	21.1(7.0)	2.2
Social/family well-being (0–28)	22.4 (3.6)	20.1(5.3)	2.2
Emotional well-being (0–24)	22.0 (3.5)	15.6(4.3)	6.7***
Functional well-being (0–28)	21.1 (2.6)	20.5(5.7)	0.6
FACT total (FACT-G) (0–108)	89.4 (10.7)	—	—
BMTS (0–40)	31.3 (6.0)	—	—

*** P<0.001.

a One patient did not complete the FACT-BMT.

b Kopp et al. [51] used the third version of the FACT-BMT, which included an additional subscale ('relationship with doctor'), relative to the fourth version that we used in the study described here. Furthermore, in the third version of the FACT-BMT, 12 items are scored in the calculation of the BMTS, whereas in the fourth version, only 10 items are scored. Therefore, it was not possible to compare our results on the FACT-G and the BMTS with those of Kopp et al. [51]

Coping

We compared the scores of BMT patients on the UCL to healthy Dutch students (n=55, ages 20–30 years) involved in a validation study of this questionnaire [53], and found that BMT patients scored significantly lower on the 'passive coping' and 'fostering reassuring thoughts' subscales of the UCL, indicating a less frequent use of these coping strategies compared to reference individuals (table 5).

Table 5. The scores of BMT patients (n=21) [a] on the Utrecht Coping List (UCL) compared to those of healthy reference individuals (n=55) [53] by means of t-tests

UCL subscales	BMT patients Mean (s.d.)	Reference individuals Mean (s.d.)	t
Active coping	17.5(3.3)	19.2 (3.7)	-1.9
Seeking distraction	16.0(3.7)	18.3 (3.1)	-2.5
Avoiding	15.3(2.1)	15.8 (3.5)	-0.8
Seeking social support	12.7(3.2)	14.9 (4.2)	-2.4
Passive coping	9.2(2.4)	12.5 (2.7)	-5.2***
Expressing emotions	6.1(1.7)	7.0 (1.8)	-2.0
Fostering reassuring thoughts	10.7(1.9)	13.2 (2.7)	-4.5***

*** P<0.001.

a One patient did not fill in the UCL.

Correlations

Patients' scores on the QOL measures (FACT-BMT, SIP, and MOS-36) were significantly correlated with each other in a comprehensible and predictable manner (table 6).

Demographic and BMT-related variables (age, sex, age at BMT, time since BMT, indication BMT, TBI dose, IQ) were not significantly correlated with patients' QOL or with their coping behaviour as assessed by the UCL, except where age at BMT was concerned. The younger the patients were at receiving

BMT, the more they are inclined at expressing their emotions as a coping strategy (r= -0.63, P<0.01). Finally, seeking social support as a means of coping with stress was significantly positively related to the emotional component of BMT patients' role functioning (r=0.63, P<0.01), whereas passive coping was significantly negatively related to patients' mental health (r= -0.67, P<0.01).

Table 6. Correlations between the measures of BMT patients' quality of life[a]

	1	2	3	4	5	6	7	8	9	10	11
SIP											
1. total score	-										
MOS SF-36											
2. Physical functioning	-0.75***	-									
3. Role functioning-physical	-0.59**	0.72***	-								
4. Bodily pain		0.69***		-							
5. General health	-0.68**	0.59**			-						
6. Vitality	-0.78***	0.68**	0.57**		0.64**	-					
7. Social functioning	-0.75***	0.60**			0.61**	0.55**	-				
8. Role functioning-emotional	-0.59**	0.63**	0.58**			0.59**	-				
9. Mental health					0.69***		0.69**	-			
FACT											
10. FACT total (FACT-G)	-0.70***	0.66**	0.70***		0.66**	0.64**	0.64**	0.73***	0.66**		
11. BMTS	-0.88***	0.65**	0.58**		0.64**	0.80**				0.75***	-

*** P<0.001, ** P<0.01,
[a] Only significant correlations are depicted in this table.

Discussion

The aim of this study was to assess the long-term effects on QOL of young adults who had undergone BMT in childhood. When compared to healthy reference individuals, the scores of BMT patients on generic measures of QOL were not significantly different from those of healthy individuals. BMT patients reported functioning as well as healthy individuals on different aspects of

health-related QOL, except where general health and overall functioning were concerned, where patients reported worse functioning. These results are in line with previous studies on the long-term effects of having undergone BMT during childhood [28;29;32].

Where work-related functioning was concerned, our patients reported suffering from some illness-related impairment (e.g. working fewer hours per week because of illness-related complaints; only being able to do easy/light chores due to illness-related impairment; only being able to work continuously for a short period of time or having to take regular breaks). In total, three participants were unemployed, of whom one was unable to work because of a physical handicap. The remaining participants were employed or fulltime students, and reported some problems in their work-related functioning, which they considered to be related to their having undergone BMT during childhood. These findings are in line with Barrera and colleagues [32], who reported that young adults, who underwent BMT during childhood, experienced more problems, compared to their peers, with regard to their studies and work possibilities.

When compared to adults who underwent BMT during adulthood, BMT patients involved in the current study, scored significantly better on a disease-specific measure of emotional well-being, indicating better emotional functioning. Despite the problems involved in comparing adult BMT recipients to childhood BMT recipients, this finding could illustrate the role of age at receiving BMT on patients' QOL. As previously reported by Schmidt and colleagues, and later by Andrykowski in the review paper, younger patients may overcome BMT-related difficulties more readily than older BMT recipients [26;56]. However, when we investigated whether demographic and BMT-related variables (e.g. age, gender, age at BMT, TBI dose) were related to patients' QOL, we found no relationships between these variables and measures of patients' QOL. Further research on QOL following undergoing BMT in childhood could shed more light on this topic.

We also investigated which strategies BMT patients adopted to cope with problems they encounter in everyday life. Patients reported coping with their problems in quite the same manner as their peers, with the exception that they reported adopting less passive coping strategies (e.g. isolating themselves from others) and fostering less reassuring thoughts (e.g. telling oneself everything will be all right). These findings could be interpreted as reflecting a

more positive and mature coping style in childhood BMT survivors relative to their peers. Similar findings have been reported in previous studies on survivors of childhood cancer [57;58]. Furthermore, seeking social support as a means of coping with stress was related to better role functioning (emotional part), whereas passive coping was related to poorer mental health. These results are in line with coping literature [59;60].

When interpreting the results, a number of limitations of this study should be kept in mind. Firstly, the cross-sectional nature of the study described here makes it impossible to draw conclusions about causality. Longitudinal studies on the long-term effects of receiving BMT are needed. Secondly, the reference groups we used in this study merit attention. We compared the scores of our BMT patient sample on the FACT-BMT to those of adult BMT recipients. This was the best reference published. Thirdly, the low response rate is another limitation of this study. As mentioned before, the main reasons for not participating were: patients attending another hospital for their regular check-ups, the time-consuming nature of the study, and the study being regarded as too physically and emotionally taxing. We did not attempt to include patients in the QOL study, described here, without them participating in the physiological measures needed for the larger study. Participating in only one section of the large study on long-term consequences of undergoing BMT during childhood was not an option. Since information gathered on non-participants via chart review showed that they did not differ from participants on demographic and BMT-related variables (e.g. sex, age at BMT, time since BMT, TBI dose; table 1), we believe that the low response rate did not affect the validity of our findings. However, participants and non-participants could have differed on other important variables such as on their way of coping with BMT. Finally, the relatively small number of participants, and the relatively large number of variables we investigated could be considered another limitation.

Notwithstanding these limitations, an important finding of this study pertains to the apparently adequate adaptation of the patients to such an intensive medical procedure as receiving BMT. Future research should focus on identifying patients at risk for developing a maladaptive response to BMT. This would help refining psychosocial support offered to BMT recipients. Studies already conducted on adult BMT recipients could be used as a model for developing studies on children receiving BMT [21;61].

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11

General discussion

Chapter 11

In this general discussion, the most important long-term endocrine effects of haematopoietic stem cell transplantation (HCT) will be discussed, combining the results presented in this thesis with other data from the literature. The first 3 paragraphs discuss growth, growth hormone (GH) secretion and effects of GH therapy, followed by 3 paragraphs on ovarian, testicular and thyroid function respectively. In the last paragraph, conclusions and recommendations are presented.

Longitudinal growth and final height in recipients of HCT

Longitudinal growth is a complex process that is influenced by genetic, metabolic, hormonal, nutritional and emotional factors. Disturbances of any of these factors can lead to impaired growth. Impaired growth has long been recognised as an important side effect of HCT [1], and many factors may contribute to this delay, e.g. anorexia, graft-versus-host disease (GVHD) of the intestine, use of glucocorticosteroids, hypogonadism, hypothyroidism, growth hormone deficiency (GHD), growth hormone resistance caused by growth plate damage. When growth ceases and the epiphyseal growth plates close, final height, the end result of longitudinal growth, is reached. The influence of impaired growth on final height depends on the capacity for catch-up growth, which is determined by both the cause and the duration of impaired growth [2].
Only recently data on final height after HCT were published (*chapters 5 and 7 included*) [3-8]. These data confirm that radiation is the major etiological factor in impaired growth after HCT, as patients who were not irradiated showed virtually no decrease in final height. In an attempt to reduce radiation-induced growth impairment, radiation-free conditioning regimens with high doses of busulphan and cyclophosphamide (Bu/Cy) have been used, especially in young children, but data on final height are limited. Most centres report no negative effect of Bu/Cy conditioning on growth several years after HCT [9-11]. However, in a small number of children without a history of cranial irradiation growth is delayed after radiation free, Bu/Cy-Based conditioning [12-14] (*see also chapter 9*). Therefore, larger populations and a longer follow-up period are needed to assess the effect of radiation-free Bu/Cy-Based conditioning regimens on growth after HCT.
Of the patients who do receive radiotherapy prior to HCT, the type of radiation most often used in conditioning for HCT is total body-irradiation (TBI). In

patients receiving TBI, final height standard deviation scores (SDS) are approximately 1 to 2 SD lower than the height SDS at the time of HCT [3;5-8] (*see also chapters 5, 6 and 10*) . In contrast to most of the earlier reports on growth after TBI [15], final height (FH) data do not support a major beneficial effect of fractionation on the reduction of adult height. Decrease in height SDS between HCT and FH reported by centres using single fraction TBI (7-8 Gy) [6;8] (*see also chapter 7*) are comparable to that reported by a large centre using fractionated TBI [7]. In addition, a large multi-centre study found no significant difference in either FH or decrease in height SDS between HCT and FH, between 39 patients receiving single fraction TBI and 39 patients receiving fractionated TBI (*chapter 5*) [5].

Radiation-induced decrease in height SDS is more prominent in patients receiving HCT at a younger age. The influence of age can be explained by the higher growth potential at younger ages. By the time final height is reached, permanent impairment of growth will therefore lead to a greater decrease in height SDS in younger children. In addition, younger children appear to be more sensitive to radiation damage, resulting in a faster decrease in height SDS in younger children (*chapter 7*) [8].

In most studies on final height, decrease in height SDS after TBI is more prominent in boys compared to girls [5;7;8;16] (*see also chapter 7*). The difference in loss of height SDS between boys and girls is attributed to blunting of the growth spurt. As the absolute height gain during the pubertal growth spurt is greater in boys than in girls, significant blunting of this growth spurt will lead to a more prominent decrease in final height SDS in boys. Another factor that could contribute to the difference in loss of height SDS between boys and girls is the timing of puberty after TBI. As hypergonadotrophic hypogonadism is much more frequent in girls than in boys [16;17], a great proportion of the girls will not enter puberty spontaneously (*chapter 6*). In these girls, puberty is frequently induced with relatively low doses of oestrogens and at a higher age compared to the mean age of spontaneous puberty in healthy girls. The late introduction of low doses of oestrogens may have a positive effect on final height.

Aetiology of impaired growth after HCT

As stated earlier, the most important contributor to growth impairment after HCT is radiation. There are several ways in which radiation may lead to

impaired growth, some of which are easily detectable and reversible (e.g. radiation induced hypothyroidism and hypogonadism). Other ways in which radiation leads to impaired growth are by damaging the hypothalamic-pituitary axis, resulting in impaired growth hormone (GH) secretion, and, probably more importantly, by damaging the growth plate, resulting in GH resistant impaired growth. Other factors contributing to impaired growth after HCT are chemotherapy induced hypogonadism, chronic GVHD and, even more important, its treatment with glucocorticosteroids.

Radiation-induced damage to the epiphyseal growth plate

Besides radiation-induced GHD, radiation-induced damage to the growth plate probably plays an important role in impaired growth after HCT as well, as impaired growth also occurs after conditioning for HCT with total lymphoid irradiation (TLI) or thoraco-abdominal irradiation (TAI), even though the hypothalamic-pituitary axis lies outside the irradiated field in these patients [5]. For obvious reasons, most data on radiation damage to the growth plates comes from animal experiments. Local irradiation of long bones with doses comparable to those used in TBI, results in massive structural damage of epiphyseal growth plates and permanent impairment of longitudinal growth [18;19]. The cause of this impairment of growth is unknown, but recent evidence suggests a role for the parathyroid hormone-related peptide (PTHrP), a paracrine/autocrine factor that co-ordinates proliferation, differentiation and structural integrity in the growth plate. Irradiation decreases the expression of PTHrP in growth plate chondrocytes both in vitro (at mRNA level) [20] and in vivo (at protein level) [21;22] (*see also chapter 3*). In addition to PTHrP, other factors will probably also contribute to the damage of the growth plate; therefore, more (animal) studies are needed to clarify the mechanisms of radiation-induced growth impairment by damage to the growth plate.

Radiation-induced damage to the hypothalamic-pituitary region

Irradiation of the hypothalamic-pituitary region can disturb the regulation of GH secretion by a yet unknown mechanism [23], with a decrease in spontaneous GH pulse amplitude but with preserved pulsatility and diurnal variation [24;25], resulting in GHD or GH neurosecretory dysfunction (GHND). In GHND the regulation of GH secretion is disturbed and spontaneous GH secretion is impaired. GH secretion in response to pharmacological stimuli, used as

diagnostic tool for GHD, however, is intact [26]. The incidence of radiation induced alterations in GH secretion increases with radiation dose and with time interval after irradiation [27;28;28-31], with disturbances at the hypothalamic level probably preceding those at the pituitary level [32].
The underlying mechanism of radiation-induced GHD is unknown. Recently a possible role for leptin in radiation induced GHD was suggested [33;34]. Apart from being a satiety signal that plays an important role in energy balance regulation, leptin is thought to influence endocrine axes, including suppression of GH secretion [35]. Brennan et al. found increased leptin levels in adults treated with cranial irradiation for childhood acute lymphoblastic leukaemia (ALL), the majority of whom had developed GHD [33]. They stated that the increase in leptin was either caused by GH deficiency or by radiation-induced damage to the hypothalamic region, resulting in leptin insensitivity. Couto-Silva et al. found a negative correlation between leptin and stimulated GH secretion after HCT and TBI, and suggested that leptin could be used as a marker for radiation induced hypothalamic-pituitary lesions [34]. Adan et al. also found increased leptin levels in patients with GHD after cranial irradiation during childhood (n=90), but in contrast to Brennan and Couto-Silva, they found no relation with GH peak response to pharmacological stimuli [36]. Whatever the cause of radiation-induced GHD, however, GH replacement therapy should result in significant catch-up growth in the absence of other causes of impaired growth (e.g. radiation damage to the growth plate).

GH secretion after HCT

There is a large variation in the reported incidence of GHD after HCT [37], which can be explained by differences in study populations such as different conditioning regimens (TBI doses, fractionation, dose rate), different proportions of patients with GVHD or a history of cranial irradiation, and differences in duration and structure of follow-up. In addition, various studies have used different criteria for the diagnosis of GHD, and many fail to use assay specific references [38]. Therefore, comparing the results of different studies should be done with caution.
Although GHD and GHND are often related to cranial irradiation prior to HCT, they also occur after TBI without a history of cranial irradiation [6;7;10;13;39-47] (*see also chapter 8*). Sanders [15] gathered information from 8 publications on the

results of GH secretion tests in 243 TBI patients (prior cranial irradiation excluded) and found that GHD was present in 64% of the patients tested, whereas we found GHD only in 9% of our TBI treated patients (*chapter 8*) [47]. Possible explanations for the low incidence of GHD in our population are the use of strict criteria for GHD [38] and the lower total TBI doses used in our transplant centre.

Radiation-free conditioning and GH secretion

As already stated, impaired growth is not a well-established complication of Bu/Cy-Based conditioning regimens, and decreased GH secretion is rare (GHD and GHND is reported in six children without a history of cranial irradiation) [13;14] (*see also chapter 9*). The high dose of busulphan (which easily crosses the blood-brain barrier) was suggested as probable cause of GHD in these patients in one report, as the plasma levels in the two patients with GHD were higher compared to other children receiving Bu/Cy [13]. Afify et al. [11], however, did not encounter impaired growth in any of their 23 Bu/Cy patients, even though their unique dosage of busulphan (150 mg/m^2 *once* daily compared to the commonly used 4 mg/kg divided in 4 gifts) will have resulted in very high peak plasma levels of busulphan. Therefore, more data are needed to establish the role, if any, of decreased GH secretion in patients conditioned with Bu/Cy.

Complications in the evaluation of GH secretion after HCT

The diagnosis of decreased GH secretion is often based on indirect markers of spontaneous GH secretion. The most important marker is the GH response to pharmacological stimuli, supported by serum levels of IGF-1 and IGFBP-3 and by auxological data such as decreased growth rate and an increased body mass index (BMI). The correlation between two consecutive GH provocation tests is poor in children treated with HCT [34], and the possibility of GHND makes GH provocation tests less sensitive in the diagnosis of decreased GH secretion. In addition, failure to use assay-specific references may result in false positive diagnosis of GHD [48]. Serum IGF-1 and IGFBP-3 levels can be used to support the diagnosis, but several studies have shown that plasma levels of IGF-1 and IGFBP-3 have only limited value in diagnosing GHD after low-dose cranial irradiation and TBI [34;36;43;49]. In idiopathic GHD, BMI is an indirect marker for the decreased GH secretion. Growth hormone, however, is only one of many factors influencing BMI; other important factors are nutritional

status and leptin. Poor nutritional status can obscure a possible effect of GHD on BMI, but in a combined European study nutritional status after TBI appeared to be good [50]. Although all indirect markers for decreased spontaneous GH secretion have a limited value in diagnosing radiation induced decreases in GH secretion, in combination (e.g. stimulated GH secretion and serum levels of IGF-1 and IGFBP-3) they could be used as an indicator. The best way to diagnose disturbances of GH secretion after cranial irradiation, however, is by constructing spontaneous GH secretion profiles, which is a costly and time-consuming method [30;51].

Value of evaluating GH secretion after HCT

The influence of GH on longitudinal growth does not only depend on GH secretion, but also on the capacity of epiphyseal chondrocytes to respond to stimuli from the somatotrophic axis. As radiation may alter this capacity to respond to the growth-promoting stimuli (sometimes referred to as GH resistance), the relation between GH secretion and longitudinal growth becomes less clear. Indeed, in our experience, GH secretion, IGF-1 and IGFBP-3 do not correspond well with either the magnitude of impaired growth or with growth response to GH therapy. Therefore, evaluation of GH secretion after TBI and HCT is not only complicated, it also has limited value in predicting the response to GH therapy, as the contribution of partial resistance of epiphyseal chondrocytes to GH is not known (chapter 8) [47]. However, GH is not only involved in longitudinal growth, but also in many other processes (e.g. bone mass, body composition, regulation of lipid- and glucose metabolism), and the effect of severely decreased GH secretion on these processes provides additional arguments for the evaluation of GH secretion. We therefore believe that GH secretion should be evaluated (using pharmacological tests) in children with impaired growth after HCT, in spite of the limited value in predicting growth response to GH therapy.

GH therapy after HCT

Effects of GH therapy

In a multi-centre evaluation of growth and final height after HCT [5], changes in HSDS between HCT and final height in patients treated with GH were similar to

the changes in patients not treated with GH. The authors' conclusion that GH therapy did not influence final height, however, is open for debate. One must assume that patients only received GH therapy on indication (e.g. more profound growth retardation or decreased GH secretion). Therefore, GH therapy was given to a selected sub-population, in which growth is more likely to be impaired, and decrease in HSDS is expected to be greater. One could argue, therefore, that similarity in height SDS changes may be interpreted as a positive result of GH treatment. Furthermore, many patients were treated at a time that GH therapy was not readily available, and GH dosages are likely to be lower than those used in the last decade, when recombinant human GH has become readily available. Most single-centre reports of GH treatment after HCT show some positive effect of GH therapy on growth. Some studies show restoration of growth without catch-up growth [45;46], whereas others show catch-up growth as well. [13;40;42]. Recently, several centres have reported final heights after GH therapy in children with a history of TBI (*chapter 8*) [47]. Using multiple regression analysis, Frisk et al. reported an estimated effect of GH therapy on height SDS of approximately 0.2 SD for each treatment year [6], whereas Sanders et al. report an estimated effect on final height of +0.86 SD [7]. In both reports children treated with GH were considered GH deficient. We recently analysed the effect of GH therapy on growth after pre-pubertal TBI in 20 patients using a random effects model, which resulted in an estimated effect of +1.2 SDS five years after initiation of GH therapy, even though 16 of them (80%) were not GH deficient.

Safety of GH therapy after HCT

Recombinant human GH is considered as a safe therapeutic agent and the general consensus is that it does not increase the risk of malignancies in patients with idiopathic GHD. We reported a relapse leukaemia in one of the 23 patients receiving GH therapy after HCT, compared to six relapses in the 43 patients not treated with GH (*chapter 8*) [47]. So far, none of the other single-centre studies on the effect of GH therapy in BMT-patients have reported a relapse of the initial disease after onset of GH therapy [6;7;13;40;42;43;45;46;52-54]. We also reported two secondary malignancies in patients treated with GH (one thyroid carcinoma and one osteosarcoma), compared to one in 43 untreated patients (malignant schwannoma) (*chapter 8*) [47]. Sanders et al. reported 6 secondary malignancies in 42 GH treated patients, compared to 8 in 48

untreated patients [7]. A large study by Sklar et al. [55] on 5-year survivors of childhood cancer revealed no evidence that treating survivors of childhood leukaemia or non-Hodgkin lymphoma with GH increased the risk of either disease recurrence or death. They did, however, observe a slight increase in the risk of secondary solid tumours (not secondary leukaemia) in patients receiving GH (n=122) compared to those who did not (n=4545), but the number of patients was small (6 solid tumours in 122 patients treated with GH; relative risk 4.98, 95% CI 1.95-12.74). In patients with other types of malignancy, no significant increase in secondary tumours was found. In addition, all six secondary solid tumours occured in patients who had received irradiation, an important risk factor for both secondary tumours and GH deficiency. Therefore, no definite conclusions can be drawn from these data, and further studies will be necessary to assess the clinical importance of these findings. It is important that the patient and/or his parents are informed about all possible risks and benefits of GH treatment before GH therapy is given to survivors of HCT.

Ovarian function

After a fixed maximum at 5 months gestational age, the number of primordial follicles in the human ovary progressively decreases with increasing age in a bi-exponential fashion, resulting in the menopause at about the age of 50 years [56]. Both chemotherapy and radiotherapy will accelerate oocyte depletion, resulting in premature ovarian failure.

Effects of chemotherapy

Radiation-free busulphan-based conditioning regimens are very gonadotoxic. In pubertal and post-pubertal females, recovery of gonadal function after Bu/Cy-based conditioning is reported in 3 of 125 women (2.4%) [57-59]. In younger women, incidences of recovery are not much higher. Combining our data (chapter 9) with several other reports [10;11;60-62], recovery of gonadal failure after Bu-based conditioning is seen in 5.3 % of 75 girls. In contrast to these data, Shah et al. conclude that long term endocrine side effects of Bu/Cy (16/200 mg/kg) conditioning for leukaemia are minimal [63]. In this study, however, only 13 of 26 relapse-free survivors (both boys and girls) were tested for ‘endocrinopathies’ and gonadal failure was reported in 2 girls using age

specific references for LH and FSH. As this report does not mention sex, age or pubertal status of the 13 patients tested, we believe the conclusions drawn by the authors are premature.

Effects of TBI

Depletion of the number of primordial oocytes after a given dose of radiotherapy is proportional to the size of the oocyte pool. As younger patients have more oocytes, the number of remaining oocytes after radiotherapy is higher in younger patients. Therefore, premature ovarian failure will occur after longer post-irradiation intervals in younger patients, giving the impression that the cytotoxic effect of radiation on the ovary is less severe in younger women [64].

Besides the age at HCT and the radiation dose, cumulative dose of gonadotoxic chemotherapeutic agents also influence the incidence of ovarian failure. The sparing effect of fractionation of TBI is unclear. As the ovary is a radiosensitive organ (radiation dose required to kill 50% of oocytes (LD_{50}) is less than 2.0 Gy) [65], the theoretical benefit of fractionation is limited, and fractionated TBI may be more damaging due to the higher total radiation dose required. In girls receiving TBI before onset of puberty, the number of patients without a spontaneous onset of puberty can be used as an indicator of gonadal failure (provided that age at TBI does not differ largely between patients). Combining the results of single centre studies that report incidences of spontaneous pubertal development after pre-pubertal TBI-based conditioning, using the most recent publication with useful information for each centre, yields the following results: the incidence of spontaneous puberty after 10 Gy sf-TBI (29 patients) is 41 % [61;66;67], whereas after 12-15.75 Gy f-TBI (63 patients) it is 43% [17;61;67;68]. In our centre, relatively low doses of sf-TBI are used (7-8Gy), which resulted in spontaneous onset of puberty in 6 of 10 (60%) patients (*chapter 6*). Frisk et al. also used 7.5 Gy sf-TBI. They reported induction of puberty in 3 of 6 girls (50%) receiving TBI before puberty [6].

In females receiving TBI-based conditioning during or after puberty, recovery of ovarian function after TBI can be used as an indicator of gonadal damage. The only study correcting for age at TBI in multiple regression analyses, reported a 4.8 times higher likelihood of recovery from initial ovarian failure after 6 x 2.0 Gy f-TBI compared to either 10 Gy sf-TBI or 7 x 2.25 Gy f-TBI [69]. The number of patients in this study, however, is limited, with recovery of gonadal function

in patients who received TBI at ages ≤ 25 years only (9 recovered: 7 of 29 after 12 Gy f-TBI; 2 of 36 after 10 Gy sf-TBI; 0 of 11 after 15.75 Gy f-TBI). In addition, the same authors reported recovery in 18 of 61 women (30%) after 8-10 Gy sf-TBI, 28 of 272 (10%) after 10-12 Gy f-TBI and 11 of 203 (5%) after 14-15.75 Gy f-TBI [15]. Unfortunately, in this report age at TBI was not reported, but unless the sf-TBI patients were significantly younger at the time of TBI, these data do not support a beneficial role of fractionation of TBI dose for gonadal function.

Based on the available data, there is insufficient evidence for a sparing effect of fractionation on the ovaries. A large (multi-centre) study, correcting for age at TBI, is needed to clarify this issue.

Testicular function

Of the three major cell types in the human testis (germ cells, Leydig cells and Sertoli cells), the germ cells are the most sensitive to cytotoxic effects of radiation and certain types of chemotherapy, especially alkylating agents, with a higher sensitivity in more primitive cell types (i.e. spermatogonia > spermatocytes > spermatids) [70;71].

Recovery of spermatogenesis occurs from surviving stem cells, and duration of azoospermia is dose-dependent and may last several years, with only partial recovery after higher doses [70;72]. There is evidence that recovery of spermatogenesis is more likely if males are treated before onset of puberty [72;73].

Effects of Chemotherapy

In men receiving radiation-free conditioning for HCT, Sanders et al. reported evidence of sperm production in 61% of patients receiving cyclophosphamide only (Cy, 200 mg/kg, n=109), and in 17% of patients receiving Cy (200 mg/kg) and busulphan (Bu, 16 mg/kg, n=46) [57]. Although the addition of Bu probably accounts for much of this difference in incidence of recovery of spermatogenesis, other chemotherapeutic agents could also contribute to the difference, as indication for HCT may have influenced the type of conditioning, e.g. Cy in patients with severe aplastic anaemia and Bu/Cy in patients with

haematological malignancies (the latter group probably had received gonadotoxic agents prior to conditioning).
Grigg et al. reported a higher incidence of recovery of spermatogenesis after radiation-free, Bu/Cy-Based conditioning with lower doses of Cy (Bu 16 mg/kg, Cy 120 mg/kg) in adults who had not received prolonged therapy with alkylating agents [58]. In this study, sperm was detectable in semen of 21 of 26 patients with semen analyses (11 had sperm count >20x10^6/ml). There was a negative correlation between serum levels of FSH and sperm count. In addition, in 6 of 21 patients without semen analyses recovery was based on successful procreation, with normal FSH levels in 4 of the 15 remaining patients. Differences in doses of cytotoxic agents, duration in follow-up (median 2 versus > 5 years), or gonadotoxic treatment prior to conditioning may account for differences in recovery between the two studies. All other studies on testicular function after radiation-free, Bu/Cy-Based conditioning have limited numbers of patients and do not report semen analysis. In our own Bu/Cy population, all 4 boys who had received low dose Bu/Cy (8 mg/kg Bu) and 2 of the 5 boys receiving high dose Bu/Cy (16-20 mg/kg Bu) with at least 2 years of follow-up during puberty, had recovery of gonadal function after Bu/Cy-Based conditioning without prior gonadotoxic treatment (*chapter 9*).
Leydig cell function is normal in most patients after Bu/Cy (*chapter 9*), although in some patients, LH is slightly elevated and serum testosterone is in the lower range of the reference interval, suggesting some Leydig cell dysfunction [10;11].

Effects of TBI

The germinal epithelium is one of the most radiosensitive human tissues, and azoospermia occurs after a single radiation dose of 0.8 Gy [74]. Complete recovery of spermatogenesis will take more than 9 months after 1 Gy, with time to recovery increasing with increasing radiation dose (e.g. > 5 years after doses of 4 Gy and higher). Both animal and human data suggest that fractionation increases gonadal toxicity of radiation [71;75]. Recently, two reviews have addressed the issue of recovery of spermatogenesis after TBI-based conditioning, and both report that the chance of recovery is small [37;76]. One review suggested a higher incidence of recovery after sf-TBI compared to f-TBI [37]. However, Sanders et al. reported comparable incidences of recovery of spermatogenesis after sf-TBI and f-TBI: 20% of 71 males receiving 10 Gy sf-TBI % and 17% of 392 males receiving 12-15.75 Gy f-TBI [57]. In theory, patients

receiving relatively low dose (7-8 Gy) sf-TBI before onset of puberty should have the highest incidence of recovery of spermatogenesis. In our study population, however, only 1 of 13 boys receiving TBI before onset of puberty showed evidence of recovery of spermatogenesis (*chapter 6*), even though 10 of 13 received 7-8 Gy sf-TBI. Three possible explanations for this low incidence are 1) the small size of the population, 2) the use of a high instantaneous dose rate (0.23 Gy/min) and 3) differences in additional gonadotoxic effects of chemotherapy prior to conditioning in our patients compared to those in the studies of Sanders et al.

Leydig cell damage occurs after a single dose of 0.75 Gy or fractionated total dose of 2 Gy in adults, resulting in transient elevation of LH but unchanged testosterone levels [74;77]. After radiation doses of 14-20 Gy to testes of patients with carcinoma in situ, Leydig cell dysfunction is often permanent [78;79], with elevated LH levels and testosterone at the lower limits of the reference interval. After doses of 18 to 20 Gy, 29% of adult patients required permanent testosterone substitution [80]. Recovery of Leydig cell function, however, can be seen after total doses as high as 24 Gy [66]. In contrast to germ cells, Leydig cells appear to be more radiosensitive in pre-pubertal males compared to adult males [73], resulting in severe hypogonadism in most boys receiving 20 Gy testicular irradiation before puberty. In our clinic, all patients who had received 10 Gy testicular irradiation in addition to sf-TBI had severe Leydig cell failure, resulting in hypergonadotrophic hypogonadism and absent pubertal development or pubertal arrest (*chapter 6* and unpublished data).

Thyroid function

Both chemotherapy and radiation can result in thyroid dysfunction, with compensated primary hypothyroidism in the majority of the patients. The incidence of thyroid dysfunction found in the different studies, however, is influenced by many other factors besides conditioning regimen, e.g. duration of follow-up, definition of compensated hypothyroidism, follow-up protocol (standard performance of TRH test at regular times versus symptoms-based screening) etcetera.

Effects of chemotherapy

After radiation-free conditioning, (compensated) hypothyroidism occurs in 10-15 % of both children [10;11;81] and adolescents [59;82-85]. In addition, Somali et al. describe exaggerated TSH responses to TRH in 61% of patients after radiation-free conditioning [85]. As they defined exaggerated response as more than 5-fold increase in TSH from baseline without any consideration for the absolute levels of TSH, this figure is likely to be an over-estimation of thyroid dysfunction in these patients.

Effects of TBI

There is some evidence that fractionation of TBI has a sparing effect on thyroid function, but reported incidences of thyroid dysfunction show a large variation between different centres. After fractionated TBI, some centres report incidences of 10-20 % [84;86] whereas others report 30-35% [87;88]. After sf-TBI thyroid dysfunction is more common, with incidences up to 87% reported by Borgström et al. [89]. In that study, the diagnosis of (compensated) hypothyroidism was based on basal values of TSH and free T4 in 48% of 27 patients after 10 Gy sf-TBI. An additional 41% had exaggerated TSH responses to TRH stimulation. Other studies using 9-10 Gy sf-TBI report incidences of hypothyroidism of 46-73 % [88;90;91].
In our single centre evaluation (*chapter 4*), the incidence of thyroid dysfunction after sf-TBI was just 11% in 18 patients. Reviewing the data of all patients in the various studies presented in this thesis, thyroid dysfunction after 7-12 Gy sf-TBI was diagnosed in 40% of 96 patients (unpublished data). These data support a role for fractionation of TBI in the preservation of thyroid function.

Conclusions and recommendations

Growth and GH secretion

In the majority of the patients receiving radiation-free conditioning for HCT, growth will not be impaired. Some patients, however, do suffer from impaired growth, and GHD could be diagnosed in few of these patients. Therefore, growth should be monitored closely after radiation-free conditioning for HCT, and further testing for GHD should be performed if growth is impaired. The use of radiation-based conditioning regimens in the preparation for HCT often leads

to impaired growth, which is a combined result of decrease in GH secretion due to radiation-induced damage to the hypothalamic-pituitary region and GH resistance due to radiation-induced damage to the epiphyseal growth plate. The value of standard tools for the diagnosis of decreased GH secretion is limited and the evaluation of GH secretion is not helpful in predicting growth responses to GH therapy. As decreased GH secretion has consequences for other metabolic processes as well (e.g. peak bone mass), Shalet et al. suggested that GH replacement therapy should be considered in all patients with GH deficiency, irrespective of auxology [92]. In children treated with HCT, GH secretion should be analysed by pharmacological tests if impaired growth is present. Due to radiation-induced growth plate damage, the results of these analyses are not related to the growth response to GH therapy, and patients with normal stimulated GH secretion may benefit from GH therapy (*chapter 8*) [47]. Therefore we would suggest that a one-year trial-period of GH therapy should be considered in all patients with impaired growth after TBI, irrespective of GH secretion status.

Gonadal function

The majority of children receiving TBI and/or high-dose chemotherapy during conditioning for HCT will suffer germ cell damage, resulting in premature ovarian failure in girls and impaired spermatogenesis in boys. Overt Leydig cell failure occurs after higher doses of irradiation and/or chemotherapy. Therefore, hypogonadism in boys is generally associated with additional gonadotoxic treatment (e.g. prophylactic testicular irradiation). We recommend routine screening for hypogonadism after HCT in all patients, with prompt initiation of sex hormone replacement therapy in cases with either hypergonadotrophic hypogonadism or delayed puberty. In view of the high incidence of infertility, we recommend attempts to preserve fertility using semen cryopreservation in all adolescent boys before initiation of gonadotoxic treatment. In girls, cryopreservation of ovarian cortical strips is an option, but expertise in both preservation and re-implantation techniques are very limited, and restoring fertility from cryopreserved ovarian tissue is still experimental [64]. In addition, in patients treated for a haematological malignancy, the risk of re-introducing malignant cells by re-implantation of ovarian tissue is of great concern.

Thyroid function

In view of the high risk of (compensated) hypothyroidism, which appears to increase with time since HCT, we recommend evaluation of thyroid function at regular intervals in all patients after HCT. Although interesting from a scientific point of view, standard dynamic testing of the hypothalamus-pituitary-thyroid axis is not recommended, as it has no therapeutic consequences. Substitution with levothyroxine is indicated in patients with overt hypothyroidism and recommended in patients with compensated hypothyroidism (the latter in an attempt to reduce the risk of thyroid malignancy and/or developmental delay) [37].

Fractionation of TBI

Endocrine late effects of HCT occur in the majority of patients receiving HCT, especially if TBI or busulphan based conditioning regimens are used. Fractionation of TBI may decrease the incidence of (compensated) hypothyroidism, but a sparing effect of fractionation is not evident for gonadal function or final height. As radiation induced thyroid dysfunction is limited to compensated hypothyroidism in most patients, and hypothyroidism is easily correctable with substitution therapy, we believe that late effects of the endocrine system do not offer solid grounds for the introduction of fractionated TBI in our clinic.

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SUMMARY

Chapter 12

This thesis contains the results of several studies on the long-term consequences of the myeloablative conditioning for haematopoietic stem cell transplantation (HCT) during infancy and childhood, with the emphasis on late effects on endocrine functions.

Chapter 1 gives a general introduction to late effects after HCT. First, the etiological roles of chronic graft-versus-host-disease and conditioning regimens are discussed, and some background information on the basic aspects of the radiobiology of total-body irradiation (TBI) is given. In addition, the most important endocrine late effects are briefly introduced.
Chapter 2 describes the effects of TBI as single toxic agents on growth, pituitary and thyroid gland in non-human primates. TBI had a negative effect on body fat. There was no evidence of (compensated) hypothyroidism, but dose dependent decrease in thyroid weight and changes in follicular structure suggest some effect of TBI on the thyroid gland. The decreased IGFI/IGFBP-3 ratio in the high-dose group may indicate that the somatotrophic axis was mildly affected by TBI.
In **chapter 3** the direct effects of radiation on the tibial growth plate of rats are studied. Radiation resulted in persistent growth delay of the irradiated tibiae, with a difference in length of more than 10% between the irradiated and the non-irradiated tibiae 15 weeks or more after irradiation. The growth plate architecture was disturbed, and the expression of both PTHrP and IHh was decreased in the irradiated tibiae. As PTHrP and IHh are key regulators of both the pace and the synchronisation of the differentiation of growth plate chondrocytes, the reduced expression of PTHrP and IHh may contribute to the changes found after irradiation.
In **chapter 4** results of a cross-sectional study on both endocrine and non-endocrine late effects of HCT during childhood are presented in a population of adult survivors of childhood HCT. Final height was reduced, with a median reduction of 1.6 SDS compared to height SDS at time of HCT as well as compared to the target height. The majority of the girls developed gonadal failure, with recovery in 2 of them. Reproductive gonadal function was decreased in the majority of the men. Hypothyroidism was diagnosed in 10% of patients. Osteopenia was found in 68% of patients, osteoporosis in 10%. Restrictive lung function was present in 67%; renal function was normal in all patients. Cataract was diagnosed in 79 % of patients who had received TBI,

the majority of these did not receive eye shielding during TBI. These data give some insight in the late effects in our centre.

Chapter 5 describes results of a large multi-centre study on final height after HCT before onset of puberty. Data on 181 patients with aplastic anaemia, leukaemia, and lymphoma were analysed. An overall decrease in final height standard deviation score (SDS) was found compared to height SDS at HCT and to genetic height SDS. Irradiation, male gender and young age at HCT were major factors contributing to decreased final height. The combination of previous cranial irradiation and single-dose TBI caused the greatest negative effect on final height. Fractionation of TBI reduces this effect significantly and conditioning with busulphan and cyclophosphamide seems to eliminate it. Nevertheless, the majority of patients (140/181) have reached adult height within the normal range of the general population.

In **chapter 6** results of a study on the pubertal development and growth of 40 children receiving TBI and HCT for haematological malignancies are presented. In the 19 boys who had not received additional testicular irradiation, penile growth and pubic hair development was normal and all had serum testosterone levels within the adult range. The majority of them, however, had incidental elevations of LH, suggesting minor Leydig cell damage. Testicular volume at the last measurement was small (mean: 10.5 ml) and serum FSH levels were elevated in all boys, suggesting severe impairment of reproductive gonadal function. Normalisation of FSH occurred in one boy. Puberty developed spontaneously in 6/10 girls who received HCT before puberty. In the remaining four girls, puberty was induced after they had developed hypergonadotrophic hypogonadism. Recovery of gonadal function after cessation of substitution was seen in one girl, who became pregnant twice (both pregnancies ended in spontaneous abortions). Decrease in height SDS was seen in the majority of patients and was positively correlated with male gender and lower age at the time of HCT. We concluded that careful monitoring of both gonadal function and growth after bone marrow transplantation and total body irradiation is warranted in order to detect disturbances early and ensure normal pubertal development in children treated for haematological malignancies.

Chapter 7 describes patterns of growth and body proportions of 75 children receiving TBI and HCT before onset of puberty. Thirty-two patients had reached final height (FH). Median change in height SDS between HCT and FH

was -1.7 in boys and -1.1 in girls. Peak height velocity (PHV) was decreased in the majority of the patients. Decrease in sitting height SDS did not differ between boys and girls (0.15 SD/yr). In boys, decrease in leg length SDS was of comparable magnitude (0.12 SD/yr), whereas in girls decrease in leg length was less pronounced (0.02 SD/yr), leading to a significant decreased sitting height/height ratio SDS in girls only. The sex-specific effects of several variables on height SDS (e.g. age at HCT, time since HCT, onset of puberty) were analysed by linear mixed-effects modelling, showing a slightly faster decrease in younger children, and a more pronounced decrease during puberty in boys compared to girls. We conclude that 1) younger children are more susceptible to growth retardation after TBI and HCT, 2) pubertal growth is more compromised in boys, and 3) leg growth is relatively less affected in girls, possibly due to a high incidence of gonadal failure in girls.

In **chapter 8** results of evaluation of growth hormone (GH) secretion in 29 children with impaired growth after TBI and HCT are presented. Impaired GH secretion was diagnosed in 8 patients, and GH therapy (1.33 mg/m^2) was started in 23 of the 29 patients tested. Height SDS increased in the first year of treatment in the vast majority of patients receiving GH (mean increase 0.35 SDS). The effect of GH therapy was estimated by comparing growth of individual patients after onset of GH therapy to the predicted growth based on individual growth before onset of GH therapy and the model derived from the study described in chapter 7. The calculated net effect of GH therapy on height SDS was +1.1 SDS. Response to GH therapy did not correlate to the parameters of GH secretion, which suggest that variable degrees of GH resistance (due to damage to the epiphyseal growth plates inflicted by the irradiation) are probably more important than GH secretion status. As GH therapy was effective in patients, irrespective of GH secretion status, we propose a trial of GH therapy in all children with impaired growth and expected low adult height after TBI.

Chapter 9 describes growth and endocrine function after busulphan (Bu) based conditioning in 64 children without a history of irradiation. Mean height standard deviation scores remained stable, but unexplained disturbances of growth after HCT 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 conditioning. Of the 49 evaluable patients, 16 developed subclinical hyperparathyroidism. We conclude that, besides gonadal and thyroid dysfunction, impaired growth and hyperparathyroidism often occur after busulphan based conditioning for HCT and that growth impairment may be the result of insufficient GH secretion.

In **chapter 10** Quality of life (QOL) is investigated in 22 adult survivors of childhood HCT, using both generic and disease-specific questionnaires. HCT-related variables were obtained from medical files. Of the generic QOL measures, most results fell within the normal range of functioning, although some illness-related impairment was reported on subscales for general and work-related functioning. Compared to a reference sample of patients who had received HCT as adults, patients involved in this study scored significantly higher on the 'emotional well-being' subscale of the disease specific QOL instrument, indicating significantly better emotional functioning. The age at HCT and TBI were not related to patients' QOL. We conclude that on the long term, having received HCT during childhood does not negatively affect the QOL of patients.

In **chapter 11** the most important long-term endocrine effects of HCT are discussed, combining the results presented in this thesis with other data from the literature. At the end of this chapter, conclusions and recommendations are given.

Chapter 12

13

SAMENVATTING

Chapter 13

Dit proefschrift bevat de resultaten van meerdere studies naar de gevolgen op langere termijn van myeloablatieve conditionering voor hematopoietische cel transplantatie (HCT) op de kinderleeftijd, met nadruk op late effecten op endocriene functies.

Hoofdstuk 1 geeft een algemene inleiding op de late effecten na HCT. De rol van chronische graft-versus-host ziekte en van de verschillende conditionering regimes wordt besproken, en er wordt achtergrondinformatie gegeven over basale aspecten van de radiobiologie van totale-lichaams bestraling (total-body irradiation, ofwel TBI). Daarnaast worden de belangrijkste endocriene late effecten van HCT kort geïntroduceerd.
Hoofdstuk 2 beschrijft de effecten van TBI als unieke noxe op de groei, de hypofyse en de schildklier van rhesusapen. TBI had een negatief effect op de hoeveelheid lichaamsvet. Er waren geen aanwijzingen voor (gecompenseerde) hypothyreoidie, maar de geobserveerde dosis-afhankelijke afname in schildkliergewicht alsmede veranderingen de folliculaire structuur van de schildklier doen enig effect van TBI op de schildklier vermoeden. De lagere IGFI/IGFBP-3 ratio in de groep apen die een hoge dosis TBI hadden gekregen kan wijzen op een beperkt effect van TBI op de somatotrope as van deze dieren.
In **hoofdstuk 3** worden de directe effecten van bestraling op de epifysaire groeischijf van de tibia van de rat beschreven. Bestraling leidde tot persisterende groeivertraging van de bestraalde tibiae, hetgeen na 15 weken resulteerde in >10% verschil in lengte tussen de bestraalde en de niet-bestraalde tibiae. De architectuur van de groeischijf was verstoord, en de expressie van zowel PTHrP als IHh was verminderd in de bestraalde tibiae. Aangezien PTHrP en IHh beide een sleutelrol spelen in zowel het tempo als de synchroniciteit van chondrocyt-differentiatie in de groeischijf, lijkt de verminderde expressie van deze para/autocriene factoren te kunnen bijdragen aan de beschreven veranderingen na bestraling.
In **hoofdstuk 4** worden de resultaten van een cross-sectionele studie naar zowel endocriene als niet-endocriene late gevolgen bij volwassenen die als kind een HCT ondergingen gepresenteerd. De mediane reductie van de relatieve lengte bedroeg bij eindlengte 1.6 standaard deviatie score (SDS) ten opzichte van zowel de lengte ten tijde van de HCT als de streeflengte op basis van ouderlengte. De meerderheid van de vrouwen had gonadaal falen

ontwikkeld, met herstel van ovariele functie in twee van hen. Reproductieve gonadale functie was verminderd in de meerderheid van de mannen. Tien procent van de patiënten had een primaire hypothyreoidie, 68% had osteopenia en 10% had osteoporose. Restrictieve longfunctieafwijkingen waren bij 67% aanwezig, nierfunctie was normaal bij alle patiënten. Van de patiënten die een TBI hadden ondergaan had 79 % cataract ontwikkeld; bij de meerderheid van deze patiënten waren de lenzen niet afgeschermd tijdens de TBI. De reslutaten van deze studie geven inzicht in de ernst en prevalentie van late effecten bij patiënten uit ons transplantatie-centrum.

Hoofdstuk 5 beschrijft de resultaten van een grote multi-center studie naar de eindlengte van kinderen die voor de puberteit een HCT ondergingen. De gegevens van 181 patiënten met aplastische anemie, leukemie of lymfomen werden geanalyseerd. Eindlengte SDS was verminderd ten opzichte van zowel de lengte SDS bij HCT als de gemiddelde ouderlengte-SDS. Bestraling, mannelijk geslacht en een lage leeftijd ten tijde van HCT waren de belangrijkste factoren die bijdroegen aan de reductie in eindlengte. De combinatie van eerdere craniale bestraling en single-dose TBI had het grootste negatieve effect op de eindlengte. Fractionering van TBI reduceerde dit effect significant en na conditionering met busulfan en cyclofosfamide leek het effect op eindlengte afwezig. Bij de meerderheid van de patiënten (140/181) lag de eindlengte binnen de normale grenzen voor de populatie (i.e. tussen –2 en +2 SDS).

In **hoofdstuk 6** worden de resultaten beschreven van een onderzoek naar de puberteitsontwikkeling en groei van 40 kinderen die een TBI en HCT ondergingen voor hematologische maligniteiten. Puberteitsontwikkeling was normaal bij de 19 jongens die geen additionele testiculaire bestraling hadden gekregen, en allen bereikten serum-testosteronspiegels binnen de referentiewaarden voor volwassen mannen. In de meerderheid was het LH echter incidenteel verhoogd, suggestief voor milde Leydig cel schade. Het testikelvolume bij de meest recente meting was klein (gemiddeld 10.5 ml) en serumspiegels van FSH waren verhoogd bij alle jongens, wijzend op een ernstige vermindering van de reproductieve gonadale functie. Bij één jongen trad normalisatie van het FSH op. De puberteit begon spontaan bij 6/10 meisjes die voor de puberteit een HCT ondergingen. Bij de overige 4 meisjes werd de puberteit geïnduceerd nadat zij hypergonadotroop hypogonadisme hadden ontwikkeld. Na het staken van oestrogeensubstitutie bleek van 1

meisje de gonadale functie te zijn hersteld. Zij werd tweemaal zwanger, maar helaas eindigden beide zwangerschappen in een spontane abortus. Bij de meerderheid van de patiënten nam de lengte SDS af met de tijd na HCT. Afname correleerde positief met mannelijk geslacht en lagere leeftijd ten tijde van de HCT. Geconcludeerd wordt dat de groei en puberteitsontwikkeling nauwkeurig gevolgd dienen te worden na HCT en TBI, teneinde verstoringen hiervan vroeg te kunnen detecteren en te behandelen.

Hoofdstuk 7 beschrijft de ontwikkeling van lengte en lichaamsverhoudingen van 75 kinderen die een TBI en HCT ondergingen vóór de start van de puberteit. Tweeëndertig van hen hadden hun eindlengte bereikt. De mediane verandering in lengte SDS tussen HCT en het bereiken van de eindlengte was -1.7 SD in jongens en -1.1 SD bij meisjes. De maximale groeisnelheid tijdens de puberteit was verlaagd bij de meerderheid van de patiënten. Afname van zithoogte SDS verschilde niet tussen jongens en meisjes (0.15 SD/jr). Afname van beenlengte SDS was van eenzelfde orde van grootte bij jongens, (0.12 SD/jr), maar bij meisjes was de afname van beenlengte SDS minder uitgesproken (0.02 SD/jr), resulterend in een significante afname van de zithoogte:lengte ratio SDS bij meisjes. De geslachtsspecifieke effecten van verschillende variabelen op lengte SDS (e.g. leeftijd HCT, tijd sinds HCT, start puberteit) werden geanalyseerd met behulp van 'linear mixed-effects' modellering. Afname van lengte SDS bleek sneller bij jongere kinderen, met een meer uitgesproken afname bij jongens ten opzichte van meisjes. Onze conclusies: 1) jongere kinderen zijn gevoeliger voor groeivertraging na TBI en HCT, 2) pubertaire groei is ernstiger gecompromitteerd bij jongens, en 3) groei van de benen is relatief gespaard bij meisjes, mogelijk ten gevolge van de hoge incidentie van gonadaal falen bij meisjes.

In **hoofdstuk 8** worden resultaten gepresenteerd van een evaluatie van groeihormoon (GH) secretie bij 29 kinderen met groeivertraging na TBI en HCT. GH secretie was verminderd in 8 patiënten, en 23 van de 29 geteste patiënten werden behandeld met GH (1.33 mg/m^2). Bij de overgrote meerderheid van deze patiënten nam de lengte SDS toe in het 1^e jaar van de behandeling (gemiddeld met 0.35 SDS). Het effect van GH therapie werd geschat door de groei van individuele patiënten tijdens GH therapie te vergelijken met de voorspelde groei op basis van individuele groei voor start therapie en het model zoals beschreven in hoofdstuk 7. Het berekende netto effect van GH therapie op lengte SDS was +1.1 SDS. De response op GH

therapie correleerde niet met parameters van GH secretie, hetgeen suggereert dat een variabele mate van GH resistentie (als gevolg van stralingsschade aan de groeischijf) mogelijk belangrijker is dan de GH secretie status. Omdat GH therapie effectief bleek bij onze patiënten, ongeacht de GH secretie status, stellen wij voor een proefbehandeling met GH te overwegen bij alle kinderen met groeivertraging en een verwachte lage eindlengte.

Hoofdstuk 9 beschrijft groei en endocriene functie na conditionering met busulfan (Bu) bij 64 kinderen zonder voorafgaande bestraling. De gemiddelde lengte SDS bleef stabiel, maar bij 17/48 patiënten (35%) zonder andere groeibeperkende factoren werd een onverklaarde verstoring van de lengtegroei na HCT gezien. (10/23 patiënten met hematologische maligniteiten). GH secretie werd geëvalueerd bij 10 patiënten, en 4 hadden een insufficiënte GH secretie. Van 52 patiënten was de schildklierfunctie beschikbaar. Twee van hen hadden een antistof-gemedieerde schildklier dysfunctie en 10 (19%) hadden een gecompenseerde primaire hypothyreoidie. De gonadale functie was beschikbaar van 21 patiënten en was normaal bij alle 7 patiënten die behandeld waren met een lage dosis Bu (8 mg/kg), terwijl 7 van de 14 kinderen die een hoge dosis Bu hadden ontvangen (16–20 mg/kg) gonadaal falen ontwikkelden. De meerderheid van deze patiënten was naast de conditionering nooit eerder behandeld met gonadotoxische therapie. Van de 49 evalueerbare patiënten ontwikkelden er 16 een subklinische hyperparathyreoidie. Wij concludeerden dat, naast gonadale en thyroidale dysfunctie, belemmering van groei en hyperparathyreoidie veel voorkomt na conditionering op basis van Bu, en dat bij sommige patiënten insufficiënte GH secretie kan bijdragen aan de groeivertraging.

In **hoofdstuk 10** wordt de Quality of life (QOL) beschreven van 22 volwassenen die op de kinderleeftijd een HCT ondergingen (patiënten uit de studie in hoofdstuk 4), waarbij gebruik gemaakt is van zowel generieke als ziekte-specifieke vragenlijsten. HCT-gerelateerde variabelen werden verzameld uit de medische registratie. De meeste resultaten van de generieke QOL metingen vielen in het normale bereik, hoewel enige ziekte-gerelateerde beperking werd gerapporteerd op subschalen voor algemeen en werk-gerelateerd functioneren. In vergelijking met een referentiegroep bestaande uit patiënten die als volwassenen een HCT ondergingen, scoorden onze patiënten significant hoger op de subschaal ‘emotioneel welbevinden’ van de ziekte-specifieke vragenlijst. Dit duidt op een significant beter emotioneel

functioneren. Noch leeftijd ten tijde van HCT, noch TBI waren gerelateerd aan de patiënt's QOL. We concludeerden dat het ondergaan van HCT op de kinderleeftijd geen negatieve invloed heeft op de QOL op de lange termijn.
In **hoofdstuk 11** worden de belangrijkste endocriene late effecten besproken, waarbij de resultaten uit dit proefschrift worden gecombineerd met overige gegevens uit de literatuur. Aan het eind van dit hoofdstuk worden conclusies en aanbevelingen gegeven.

CURRICULUM VITAE

The author of this thesis was born on October 4th 1967 in Dubbeldam, the Netherlands. He attended secondary school at the 'Johan de Witt Gymnasium' in Dordrecht and passed his gymnasium β exams in 1986. After studying biomedical sciences at Leiden University for one year, he started his medical training in 1987 at the same university. During medical training the research on late effects of total-body irradiation (TBI) and haematopoietic stem cell transplantation (HCT) was started at the department of oncology of the Leiden University Medical Centre (supervisor Prof.Dr. J.J. Broerse). After obtaining his medical degree in 1995, he continued his research on late effects of TBI and HCT at the department of paediatrics of the Leiden University Medical Centre (head and supervisor Prof.Dr. J.M. Wit). In 1997 he obtained a grant from The Netherlands' Organisation for Scientific Research (NWO-AGIKO stipendium), enabling him to combine his research activities with his training in paediatrics at Leiden University Medical Centre (Prof.Dr.J.M. Wit) and Reinier de Graaf Gasthuis in Delft, (Dr. N van der Lely). In November 2004 he started his training in paediatric endocrinology at the Leiden University Medical Centre (tutor Prof.Dr. J.M. Wit).

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