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

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General introduction

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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