Optimization of total body irradiation: the match between (maximal) leukemic cell kill and (minimal) late effects

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Optimalisatie van totale lichaamsbestraling:
de queeste naar (maximale) vernietiging van
leukemische cellen en (minimale) late schade.

(*met een samenvatting in het Nederlands*)

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GENERAL INTRODUCTION

Chapter 1

INTRODUCTION

Bone marrow transplantation (BMT) has in the past been closely linked to intentional or accidental irradiation. Initial investigations were driven by attempts to understand the hematopoietic syndrome that occurred in men exposed to the atom bombs in Hiroshima and Nagasaki in 1945 (1). Jacobsen was the first to demonstrate that mice could survive lethal radiation doses by shielding the spleen (2). At first was thought that the protection was due to humeral factors. In 1951, Lorentz reported that infusion of bone marrow cells could prevent the hematopoietic syndrome (3). In 1957, Thomas et al. reported about patients to whom infusion of bone marrow cells was applied (4). These first experiments showed that radiation severely damaged bone marrow cells and that normal stem cells were able to repopulate the bone marrow.
In the next decade little progress was made. Results were initially disappointing. In the 1950^{s} and 1960^{s} almost all attempts to achieve allogeneic grafts in humans were unsuccessful. Rejection of the graft, infections and graft versus host disease (GVHD) were major problems. A few patients, however, although terminally ill, were cured and an intensive research started for the solution of the problems. Progress in the knowledge of the histocompatibility typing, anticipating and management of graft versus host disease (GVHD) and supportive measures for patients with no marrow function led to the resumption of the attempts to carry out marrow transplantation towards the end of the 1960^{s}.

When was discovered that the number of peripheral blood stem cells could be increased by the administration of hematopoietic growth factors (5), the use of hematopoietic stem cells (HSCs) from sources other than bone marrow started. The terminology shifted from bone marrow transplantation (BMT) to hematopoietic stem cell transplantation (HSCT) (6).
From a merely technical point of view, HSCT is one of the easiest organ transplantations, in contrast to the complexity of the involved immunological mechanisms. Operationally there are six consecutive steps (1):

1. Patient selection or indication
2. Histocompatibility testing and donor selection
3. Ablative “conditioning” of the patient’s hematopoietic and immune system
4. Marrow replacement
5. Control of the graft-versus-host disease
6. Control of complications, late effects and relapse

Chapter 1

1.1 PATIENT SELECTION OR INDICATION

Marrow transplantation is a treatment option for a patient when the disease involves the bone marrow or when the aggressive treatment of a disease is limited by hazard to the normal bone marrow (1). Diseases treated with marrow transplantation are hematological malignancies, severe aplastic anemia, immunologic deficiency diseases, enzyme deficiency diseases in case of lack of an enzyme, normally produced by bone marrow derived cells, and some of the solid tumors for which the therapy to cure is limited by marrow toxicity. Indications for BMT changed through the years. Main indications in 2004 in Europe were leukemias (32%), lymphomas (55%), solid tumors (8%) and non-malignant disorders (5%) (7). The remission status of a patient submitted for transplantation is of importance. Age can be a limiting factor, although age limits also changed through the years, especially since reduced intensity conditioning (RIC) is employed. In practice all centers for hematological stem cell transplantation in The Netherlands have fixed protocols for treatment and patient selection, although the protocols may differ per center.

1.2 HISTOCOMPATIBILITY TESTING AND DONOR SELECTION

1.2.1 Histocompatibility testing

In 1958, antibodies, induced by transfusions or pregnancy (8) and reacting with antigens on white blood cells, were used to describe human leukocyte antigens (HLA) groups (9,10). Now it is known that these antibodies provoke immune reactions when tissues are grafted from one individual to another. The genetic control of these antigens resides on chromosome 6 in a gene region known as the major histocompatibility complex (MHC). In this region, class I antigens involve three loci important in transplantation: HLA-A, -B, and –C. Class II antigens are governed by HLA-DR, -DP, and-DQ. These regions constitute a “haplotype” (11). Each parent has two haplotypes, and each child inherits one haplotype from each parent. Thus there is one chance in four that a patient will match with his/her brother/sister

In the 1970^{s} and 1980^{s} most bone marrow transplantations involved sibling donor pairs. In the 1990^{s} serologic typing techniques have been replaced by better, molecular techniques (6). Also the matching of unrelated individuals became possible. Success of BMTs between matched unrelated individuals now approximates that between matched unrelated siblings. Almost half of allogeneic HSCTs are now from unrelated donors (12).

1.2.2 Donor selection

A graft can be autologous, derived from the patient's own stem cells, syngeneic, from the stem cells of a genetically identical sibling and allogeneic, when donor and recipient are of different genetic origin, related or unrelated (1)

Autologous grafts are used in neoplasms without and with bone marrow infiltration after cytotoxic therapy (e.g. solid tumors and lymphomas) but also in neoplasms of the bone marrow itself after clinically eliminating the malignant clone. Allogeneic grafts are preferred in diseases that involve the bone marrow, but there are many reasons to deviate from this rule. In 1999, 70% of all leukemia's were transplanted allogeneic, 92% of all transplanted lymphomas were transplanted autologous, 99% of all solid tumors were transplanted autologous and 85% of all nonmalignant disorders were transplanted allogeneic (13). Recently an increase of allogeneic HSCT was reported and an increasing use of unrelated allogeneic HSCT, which in 2005 comprised in Europe 45% of all allogeneic HSCTs (12).

1.3 ABLATIVE CONDITIONING OF PATIENT'S HEMATOPOIETIC AND IMMUNE SYSTEM

In the early years, ionizing radiation was a logical choice as a conditioning regimen as leukemic cells were known to be highly radiosensitive. Because most patients had advanced leukemia and were very ill, single-exposure TBI was applied to establish a graft. In Seattle, 1000 cGy TBI was administered at 7 cGy/min (6). Cyclophosphamide (Cy) was known as an antileukemic agent and reported to be an immunosuppressive agent (14). In Seattle, Cy was combined with TBI and this regimen produced the first long-term disease-free allogeneic patients (15). In later years, fractionation of the TBI became feasible and also other preparative regimens were tested. Nowadays, many transplant teams apply radiation as a single dose or as a series of fractionated doses and chemotherapeutic agents in various combinations (6).

The most commonly used myeloablative regimen still is cyclophosphamide 60 mg/kg for two consecutive days followed by TBI, administered fractionated or as a single dose (16). There is, however, no clear evidence yet that any combination of drugs and irradiation is more effective than another. The preparative regimen is directed at ablation of hematopoietic elements, suppression of immunological resistance and, in patients with malignancies, eradication of residual malignant cells.

The standard high dose conditioning has been challenged in the recent years by RIC. This

form of conditioning only paves the way for donor engraftment with immunosuppressive agents; the effect of the treatment relies exclusively on immunological mechanisms. A large variety of RIC regimens is in use. Low-dose TBI can be part of it, generally applied in one fraction of 2 Gy

1.3.1 TBI

In TBI, the target volume consists of the patient as a whole and the dose distribution should be as homogenous as possible, despite variations in thickness and non-uniformity. As the generally used TBI doses are relatively high, it is necessary to keep the dose applied to some critical organs below their tolerance dose.

To fit the patient into the field generally a maximal field size and large treatment distances are used, while the patient is positioned along the diagonal of the square of the field with rotated collimator. The patient is irradiated with anterior and posterior (AP/PA) fields or with parallel opposing lateral fields. Using AP/PA fields, the patients is positioned along the horizontal diagonal of the rotated field lying on his side with bended knees, or along the vertical diagonal of the field, sitting upright in a specially designed chair, also with bended knees. Using lateral field irradiation the patient is situated in a chair, supine, in a sitting position.

Inhomogeneity of the dose distribution can be decreased by the use of high-energy photon beams. There is, however, a substantial build-up at the surface, causing underdosage of the skin. In most centers this is compensated for with a slab of tissue-equivalent material near to the patients surface. Also at places with decreased thickness (e.g. the neck) often tissue equivalent bolus material has to be used for compensation (17).

To keep the dose to critical organs below their tolerance dose the possibilities are: 1.Fractionation, 2. Lowering of the dose-rate, 3. Decreasing the dose locally by shielding of the organs concerned, or 4. A combination of the first three possibilities. Using one- or two-fraction regimens at a relatively high dose-rate as applied in The Netherlands, shielding is the only way to avoid exceeding the tolerance dose of organs at risk. Fractionation is reported to lower the impact of the TBI on normal tissues, without affecting the effect on leukemic cells (18). Some shielding remains, however, unavoidable, also in fractionated TBI.

Several protocols have been designed for fractionated irradiation. In general, higher doses seemed to reduce the risk of relapse and rejection but to increase the risk of transplant-related mortality (19). In practice, centers selected their schedules initially on the basis of local conditions such as available treatment room, treatment machine and patient load; many

different TBI schedules are currently still in use without convincing evidence for the superiority of their selection (18). Shielding is one of the major variables.

1.3.2 Linear quadratic (LQ) model and biologic effective dose (BED)

The aim of radiotherapy is to kill cancer cells while damage to the normal tissues is limited. The expected effects, to the tumor and any other irradiated organs, therefore, need to be known.

Although the LQ model had been available for several years it was not used by the radiotherapy community until 1982 (20). The introduction of the concept of the extrapolated response dose (ERD) by Barendsen (21) provided the simplicity for common use. ERD was later renamed as biologic effective dose (BED) by Fowler (22). To be able to predict the impact of whatever radiotherapeutical treatment, the LQ model can be used to consider the variation in tissue response with fraction size. One of the main uses of the LQ model is, normalizing fractionation schedules and, therefore, being able to compare different fractionation schedules.

The BED is a parameter derived from the LQ model and is based on the concept of the effects of irradiation, as it represents the calculated dose causing an equivalent effect for an infinite number of fractions (20, 21). According to the LQ-BED concept, the occurrence of a biologic effect (e.g. cataract-incidence) depends on the dose in a linear and quadratic fashion $E = n (\alpha d + \beta d^2)$ were n is the number of fractions, d is the dose per fraction and α and β are constants (21, 23). From this equation, the BED can be derived: $BED = nd [1 + d/ (\alpha/\beta)]$.

When treatments are considered, requiring to take also into account the process of repair and the dose rate of the irradiation (brachytherapy and low dose rate hyperfractionated schemes), LQ-models are used considering also dose rate and a time constant relating to the rate of sublethal damage repair, μ.

The strength of the LQ concept is, that for all treatments the BED values that result in a specific effect (e.g. a certain percentage of injury on healthy tissue), are equal.

The value of α/β is about 10 Gy for acute reacting tissues like leukemic cells and 1-4 Gy for late reacting tissues. The rate of sublethal damage repair μ is about 1 h^{-1} for acute reacting tissues and 0.3-0.9 h^{-1} for late reacting tissues (23). For each TBI schedule a specific BED can be calculated for the leukemic cells as well as for late responding normal tissues, provided that the α/β and μ values of the concerning tissues are known. This allows comparing of the effects of the different TBI regimens.

1.4 MARROW REPLACEMENT

Stem cell source has changed from almost completely bone marrow in 1990 to peripheral blood as the main source of stem cells in 2004, with a few exceptions especially for allogeneic HSCT for non-malignant disorders (7).
Bone marrow stem cells for transplantation are generally harvested from the pelvic bones of patients or donors. After specific working up into a suitable specimen they are administered to the patients by intravenous infusion. Peripheral blood stem cells (PBSCs) are harvested from the peripheral blood after administration of hematopoietic growth factors. Red cells or plasma are removed in the case of ABO incompatibility, T cells can be removed to prevent graft versus host disease (GVHD). The repopulating stem cells find their way by a phenomenon called 'homing'. Subsequently they seed the marrow cavity and restore bone marrow cellularity within a period of three to four weeks. Eventually, the entire hematopoietic and immune system is replaced and becomes of donor origin. Months to years are needed for full recovery of the immune competence. GVHD influences the recovery of the function of the immune system. In the immediate peritransplant period, red cells and platelet transfusion must be given to substitute for the absent bone marrow. Also antibiotics, antifungal, and antiviral therapy are required to protect the host from infections. Most patients are kept under some form of isolation for some time. Furthermore, often intravenous feeding is required (1).

1.5 GRAFT VERSUS HOST DISEASE

Acute GVHD is the consequence of immunocompetent engrafted donor lymphoid elements, recognizing host tissue antigens different from their own. It occurs within the first 100 days after transplantation and is characterized by skin rash, hepatic dysfunction, diarrhea and fever. Chronic GVHD is a multi-system disorder, maximally expressed between 100 and 400 days post transplant (1).
Extensive studies in mice have defined the role of T cells and of histocompatibility systems in the GVH reaction (24). In the early 1970s, when the donor was a HLA-matched sibling, GVHD occurred in half of the patients. Prevention, or treatment with methotrexate (MTX) and glucocorticoids, was only partially effective (25). Cyclosporine improved the prevention of GVHD (26). Later on, the amelioration of GVHD by the removal of T-cells was described. It is now known that removal of T-cells from the graft can prevent GVHD,

although it increases graft failure, delays immune recovery and diminishes the graft-versus-leukemia (GVL) reaction (27). Some form of T-cell removal is, however, applied in most centers.

Clinically, GVHD is separated in an acute and a chronic form, based on clinical manifestation, time interval after BMT and histology. Chronic GVHD can develop in connection with the continuing active form, after an interval when the active manifestation has been disappeared, and de novo. The most commonly applied immune suppressive agents are cyclosporine, methotrexate (or the combination of both) and steroids.

The existence of a GVL effect - donor cells capable of destroying residual leukemic cells by a reaction of immunity, separated from a GVH reaction - has in 1979 been described in mice (28) and in men (29).

In the late 1980th, investigators began to explore the possible antileukemic effect of donor lymphocyte infusions for relapse after transplantation (30,31). It was shown that donor lymphocyte infusions on their own could induce long-lasting remissions (32). The antileukemic effect of donor lymphocyte infusions was confirmed in numerous reports, with risks to host marrow suppression and GVHD. Studies in dogs and humans demonstrated that it is possible to get marrow grafts with less toxic regimens if combined with sufficient immune suppression and generous infusion of hematological stem cells (HSCs), resulting in mixed grafts of host and donor cells (33,34). This was the beginning of the allogeneic transplantations using RIC regimens.

1.6 CONTROL OF COMPLICATIONS, LATE EFFECTS, AND RELAPSE

1.6.1 Acute effects and complications

Acute effects after HSCT are the results of the conditioning therapy that is toxic and leaves the patient without functioning marrow cells. Acute side effects of the conditioning regimen with cyclophosphamide and TBI are rash, nausea vomiting, fever, hemorrhagic cystitis, carditis, parotitis, diarrhea and alopecia.

Many undesired effects after transplantation are of multifactorial etiology.

The potential benefits of allo-HSCT-mediated graft-versus-tumor effects are limited by the morbidity and mortality associated with GVHD and its associated immune deficiency state. Whereas the prophylactic use of multiagent immunosuppression has reduced the incidence and severity of acute GVHD, the incidence of chronic GVHD (cGVHD) remains unchanged. Chronic GVHD and the associated immune deficiency is a prime cause of transplant-related-

mortality after allogeneic HSCT and contributes directly or indirectly to most complications (35).

The depletion of the host immune system and the delay in immune reconstitution predispose to the development of opportunistic infections that are among the leading causes of death following HSCT. Better diagnostic tools and novel antibiotic drugs, however, have reduced dramatically the morbidity and mortality from infections associated with HSCT in the recent years (16,36). Guidelines for preventing and treating opportunistic infections after HSCT are proposed and published (37).

Closely linked with GVHD is interstitial pneumonitis, which, for a large proportion, is of infectious (viral) origin. In most of the remainder the process is idiopathic, probably representing the combined adverse effects of damage by irradiation, drug toxicity and GVHD (1).

1.6.2 Late effects

Usually, complications are considered as late effects after a follow-up of 6 month after HSCT.

Lung.

Late pulmonary effects are divided in restrictive lung disease, chronic obstructive pulmonary disease and obliterative bronchiolitis (35). Restrictive lung disease is in most cases not symptomatic. It is often stable and may recover within 2 years (38, 39). Chronic obstructive lung disease is mainly associated with cGVHD, but other potential risk factors have been described. Mortality is high, particular in those with an early onset (35). Obliterative bronchiolitis is mainly associated with cGVHD, caries a mortality rate of 50 % and does not respond to steroids (35).

Eye.

Ocular complications of the posterior segment of the eye can be divided in microvascular retinopathy, optic disc edema, hemorrhagic complications and infectious retinitis. Microvascular retinopathy is a multifactorial process and occurs mainly after TBI-conditioned allogeneic HSCT in patients receiving cyclosporine. The lesions mostly dissolve after withdrawal of the immune suppressive therapy (35).

Ocular complications of the anterior segment are keratoconjunctivitis sicca syndrome and cataract formation. Keratoconjunctivitis sicca is part of a Sjögrenlike syndrome. The incidence may reach 40% in patients with cGVHD (35). Cataract is every detectable change is the normally transparent eye lens. The form of cataract associated with ionizing

irradiation is posterior capsular cataract (40, 41). In multivariate analysis, the use of TBI, including total dose, number of fractions and dose rate of the TBI, and the use of steroid treatment for > 3 month, are associated with a significant risk of cataract development (42, 43, 44). Cataract surgery nowadays is a low risk procedure and restores vision in 95% of the eyes that have no other pathology.

Kidney.

TBI is by several authors reported to be the principal factor for late renal dysfunction (45, 46, 47, 48) and mainly attributable to radiation nephropathy characterized by signs of hypertension, edema, anemia and hematuria, with elevated serum creatinine concentration and decreased glomerular filtration rate (49). Some institutes use selective renal shielding blocks (48, 50, 51).

Igaki et al. found that renal shielding reduced the renal toxicities without decreasing the overall survival (48). Miralbell found that only TBI and GVHD were significantly correlated with late renal dysfunction (51).

Thyroid.

Thyroid dysfunction after HSCT can be classified into three distinct patterns: subclinical compensated hypothyroidism, overt hypothyroidism and autoimmune thyroid disease.

Subclinical compensated hypothyroidism develops in 7-15.5% of patients and may resolve spontaneously (52, 53). It is not clear if patients should be treated with L-thyroxin. Overt hypothyroidism is relatively rare. The frequency varies depending on pretransplantation conditioning and age of the patient (54), with a greater risk among younger children (55). Autoimmune thyroid disease may be transferred via donor cells (56), although it is also reported in patients irradiated for Hodgkin's disease, who had no HSCT. So also other mechanisms may play a role.

Liver.

Unraveling the cause of liver dysfunction can pose difficulties, because several causes of liver disease may coexist. Tools for differential diagnosis are time of appearance post transplantation, type of clinical and biochemical deterioration, previous evidence of liver complications including veno-occlusive disease, GVHD, and infection. Most important hepatotropic viruses are the hepatitis B and hepatitis C viruses.

Another common cause of liver dysfunction is iron overload that is diagnosed in up to 88% of the long-term survivors of HSCT (35).

Growth.

Linear growth is a process that may be affected by genetic, nutritional, hormonal and psychological factors. Intensive anticancer therapy during childhood may influence these factors resulting in decreased growth (35). Final height was found to be compromised because of blunted growth in puberty in patients treated in childhood with chemotherapy and TBI for HSCT (57). It was significantly correlated with age at diagnosis, age at TBI and target height (58). Fractionation of the TBI reduced the effect and conditioning with chemotherapy alone seemed to eliminate it (59).

Puberty and gonadal failure.

Gonadal failure is a common long-term consequence of the chemotherapy given prior to HSCT and of the pretransplant conditioning. In males, the germinal epithelium is more vulnerable to radiation and chemotherapy than the Leydig cell component, which is involved in the testosterone secretion. Therefore, testosterone levels are generally normal. Male long-term survivors, who were younger than 25 years at HSCT and had no cGVHD, have a reasonable likelihood of spermatogenesis even when conditioned with TBI (60). In females, the ovaries are more vulnerable to irradiation and chemotherapy than the testes and hypergonadotropic hypogonadism is almost a rule (35).

After TBI and BMT, pubertal development is normal in most boys, although there are subtle changes in Leydig cell function and spermatogenesis is likely to be severely affected. In approximately 50% of the girls treated before puberty, pubertal development and gonadal function are normal. Gonadal insufficiency can be expected in the vast majority of those girls treated after the onset of puberty (61).

Bone and joints.

Among late complications of bone and joints is avascular necrosis of bone and joints. The use of steroids (total dose and duration) is the strongest risk factor for this complication. The second major risk factor is TBI (62). Another complication is osteoporosis that is significantly associated with glucocorticoid therapy and the duration of cyclosporine and tacrolimus therapy. Preventive measures of osteoporosis should include sex-hormone replacement in patients with gonadal failure (35).

Carcinogenesis.

Radiation and chemotherapy can cause genetic damage and increase the carcinogenic risk after autologous BMT (63). Immunosuppression therapy exerts a co-carcinogenic effect that favors the outgrowth of malignant cells. The role of the immune system clearly is extremely important. Generally the problem of second malignancies is divided into three groups, i.e. leukemia, lymphoma and solid tumors. Most post-transplant lymphoproliferative disorders are associated with allogeneic HSCT, T-cell dysfunction and the presence of EBV (64) and occur within a median elapsed time of 2.5 month. Second leukemia's occur after a median period of 6.7 month, while the median elapsed time for solid tumors lies between 5 to 6 years (64). Risk factors reported for the development of solid tumors are TBI, treatment for chronic GVHD, and older age (65,66). Rizzo et al reported about the solid cancer risk in 28 884 patients surviving 10 years or more after transplantation. The average age at transplantation was 27 years (67). They found the cumulative incidence of solid cancers for all patients to be 2.2% at 10 years, 5.0 % at 15 years and 8.1% at 20 years. Relative risk was 4.8 among 10-year survivors and increased with time. Sites with significantly increased risks were oral cavity, skin, thyroid, salivary glands, brain, liver and bones. For breast cancer there was a significantly increased risk among 10-year survivors. For thyroid carcinoma, young age at transplantation is the most important risk factor, followed by irradiation, female sex and cGVHD (68). The most common reported second cancers were cancer of the oral cavity and squamous cell carcinoma of the skin.

1.6.3 Relapse

Relapse of the disease is now the most common cause of treatment failure after allogeneic HSCT. The lowest probability of relapse was seen in patients who are in first complete remission of AL or in first chronic phase of CML: 10-30% (69).

In second or later remission, 40-50% of the patients relapse, and in patients with advanced disease the incidence may be in excess of 70%. T-cell depletion of the graft and also more effective immunosuppression, for instance by combining MTX and CsA, increases the risk of relapse. Severe GVHD should, however, be prevented. Best survival is seen with mild acute and with chronic GVHD.

In general prognosis of patients who relapse is poor and an optimal salvage therapy has not been established yet. Current treatment options include chemotherapy, donor leukocyte infusion (DLI) and second allogeneic transplantation. Chemotherapeutic regimens can induce remissions in some patients, but remission durations are short (70). Results of DLI in patients with AL are not encouraging: 10-20% complete remission while

remissions are mostly temporary. Only CML patients have a good prognosis after DLI (71, 72).
Second allogeneic transplantation may be an effective salvage therapy in some patients with relapse after an initial transplant and can produce sustained remissions in a proportion of the patients. Disease status, age, conditioning regimen and duration of remission after first transplantation are the most important determinants of outcome. A second transplantation is of no use with remission durations of ≤ 6 month (70).

1.7 THIS THESIS

In this thesis cataract formation and renal dysfunction as late effects of high-dose TBI before HSCT, are considered in more detail. Discussed are factors influencing their appearance, as well as, in case of cataract formation, the final severity of the lesion and complaints resulting from it. Also possibilities to prevent severe cataracts, late renal dysfunction and late effects of high-dose TBI for HSCT in general are investigated or discussed. With respect to cataract formation, the values of the tissue specific parameter α/β for lens tissue and the rate of repair of sublethal damage μ are derived. They were unknown yet and are required to calculate the cataract incidence for a specific irradiation regimen, using the LQ-concept.
Furthermore, a study is performed to find the requirements of an optimal TBI regimen with a maximal leukemic cell-kill and minimal late effects. Finally, the results of HSCT/TBI in The Netherlands are discussed, with special attention for the effectiveness of the various TBI regimens, while also the effects of other variables are considered such as age, gender and type of transplantation.

1.7.1 The aim of the thesis is to answer the following main questions:

- What are the characteristics of cataract development after TBI in one or two fractions as part of the conditioning for HSCT, and what are the consequences of a radiation cataract with respect to the visual impairment.
- Is there a risk involved in preventively shielding the eyes during TBI.
- Is it possible to predict the incidence of cataract formation for a specific TBI regimen.
- What is the tolerance dose for kidney tissue when TBI as part of the conditioning for HSCT is applied.

- What are the requirements for an optimal TBI regimen concerning a maximal leukemic cell kill and OS and minimal late effects on lungs, kidneys and eye lenses.
- What are the results of HSCT/TBI in The Netherlands.

1.7.2 Outline of the thesis

In **Chapter 1** topics, dealt with in this thesis, are discussed.

In **Chapter 2** probability of cataract formation after TBI as part of the conditioning for HSCT, latency time of the cataract and grade of severity a cataract finally attains, are prospectively studied.

In **Chapter 3** the degree of visual impairment, resulting after formation of stable cataract are described. Probability of cataract formation and the probability developing of severe visual impairment are compared.

In **Chapter 4** risks and benefits of eye shielding during TBI in 188 children are retrospectively investigated.

In **Chapter 5** the determination of a dose-effect relationship for cataract induction is presented. Using data, collected by the European Group for Blood and Marrow Transplantation for an earlier investigation of cataract development after different TBI regimens, we could derive, α/β and μ values for lens tissue, that were previously unknown.

In **Chapter 6** a dose-effect relationship is derived for the endpoint late renal dysfunction after TBI, by the comparing and analysis of literature derived data.

Chapter 7 describes the results of a study in search of the requirements a TBI regimen has to fulfil, to obtain optimal results with respect to leukemic cell kill, overall survival and late effects on normal tissue.

Chapter 8 describes the results of a retrospective study in five centers in The Netherlands investigating the influence of TBI regimen, age, gender, type of transplant and type of disease on the endpoints overall survival, relapse-incidence, transplantation-related-mortality and disease-free survival. Attention was also paid to the effect of dose reduction by shielding blocks.

Chapter 9 contains a summary of the thesis, results, general discussion and recommendations.

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Chapter 2

CATARACT-FREE INTERVAL AND SEVERITY OF CATARACT AFTER TOTAL BODY IRRADIATION AND BONE MARROW TRANSPLANTATION: INFLUENCE OF TREATMENT PARAMETERS

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ABSTRACT

Purpose: To determine prospectively the cataract-free interval (latency time) after total body irradiation (TBI) and bone marrow transplantation (BMT) and to assess accurately the final severity of the cataract.

Methods and Materials: Ninety-three of the patients who received TBI as a part of their conditioning regimen for BMT between 1982 and 1995 were followed with respect to cataract formation. Included were only patients with a follow-up period of at least 23 months. TBI was applied in one fraction of 8 Gy or two fractions of 5 or 6 Gy. Cataract-free period was assessed and in 56 patients, who were followed until stabilization of the cataract, final severity of the cataract was determined, using a classification system. With respect to final severity, two groups were analyzed: subclinical low-grade cataract and high-grade cataract. Cataract-free period and final severity were determined with respect to type of transplantation, TBI dose, graft versus host disease (GVHD) and steroid treatment.

Results: Cataract incidence of the analyzed patients was 89%. Median time to develop a cataract was 58 months for autologous patients. For allogeneic patients with or without steroid treatment, median times were 33 and 46 months, respectively. In allogeneic patients, however, final severity was significantly different for patients who had or had not been treated with steroids for GVHD: 93% versus 35% high-grade cataract, respectively. Final severity was also different for patients receiving 1 x 8 or 2 x 5 Gy TBI, from patients receiving 2 x 6 Gy as conditioning: 33% versus 79% high-grade cataract, respectively. The group of patients receiving 2 x 6 Gy comprised, however, more patients with steroid treatment. The high percentage of high-grade cataract in the 2 x 6 Gy group might have been caused by steroid treatment. The percentage of patients with high-grade cataract was lower in allogeneic patients without steroid treatment than in autologous patients: 35% versus 48%. An explanation for this could be pretransplant therapy containing high dose steroids.

Conclusions: After high dose rate TBI in one or two fractions, steroids for GVHD influence latency time of cataract and are of great importance for the severity the cataract finally attains. Although a cataract will develop in all patients, a clinically important high-grade cataract is relatively infrequent in patients not treated with steroids

INTRODUCTION

Bone marrow transplantation (BMT) is a widely applied treatment modality for patients with hematological malignancies and aplastic anemia (1,2). Total body irradiation (TBI) is an important part of the conditioning regimen (1-4). Cataract formation has been recognized as one of the most frequent late complications of TBI (5-7). Because the orbit is regarded as a possible site of recurrence of disease, it is generally not shielded. Of particular concern are posterior capsular cataracts as this form of cataract is associated with ionizing radiation (8).

TBI parameters and other factors such as steroid medication, graft versus host disease (GVHD), and prophylactic cranial irradiation have been reported to influence cataract formation (5,7,9,10). When regular ophthalmologic follow-up is performed, cataract is often detected in an early subclinical stage. Subsequently, it progresses for some time and then stabilizes in a certain grade of severity (11,12). In institutions for radiotherapy in The Netherlands, single dose TBI (STBI) or TBI in two high dose fractions is applied and cataract can therefore be expected to appear in the majority of patients (5,10). However, the grade in which a cataract finally stabilizes appeared to vary largely and with that the long-term visual problems.

In this report factors are analyzed that influence the length of the cataract-free period after BMT and the final severity of a cataract, using a classification system by which we are able to determine accurately the course of the cataract and its final severity.

METHODS AND MATERIALS

Patients

Ninety-three of the patients who received TBI as a part of their conditioning regimen for autologous (n = 42) or allogeneic (n = 51) BMT between 1982 and 1995 were followed. As there were many patients with severe transplant-related toxicity or early relapse after BMT, and as in our series only few (n = 3) patients developed a cataract within the first 2 years after BMT, only patients with a follow-up period of at least 23 months were included. TBI was administered in one single-dose fraction or two fractions on two consecutive days.

Table 1 gives patient characteristics (age, gender, diagnosis); Table 2 gives characteristics of the treatment (dose, fraction size, type of bone marrow transplantation). The median follow-up period was 74 months (range 23-161).

Table 1: Age, gender and diagnosis of 93 patients

Age (years)	Median	35 (range 14 – 56)
Gender	Male	38
	Female	55
Diagnosis	AML	18
	ALL	18
	CML	11
	NHL	32
	Multiple Myeloma	7
	Aplastic Anaemia	7

Abbreviations: AML = Acute Myeloid Leukemia; ALL = Acute Lymphoid Leukemia; CML = Chronic Myeloid Leukemia; NHL = Non Hodgkin's Lymphoma.

Table 2: Treatment doses, fraction size and type of BMT

Autologous BMT		
1982 – 1995	1 x 8 Gy	(n = 42)
Allogeneic BMT		
1982 – 1987	1 x 8 Gy	(n = 8)
1987 – 1989	2 x 5 Gy	(n = 11)
1990 – 1995	2 x 6 Gy	(n = 32)

Conditioning Regimen

All patients were conditioned with high-dose chemotherapy (CT) and TBI. Chemotherapy consisted of cyclophosphamide 60 mg/kg/day and was administered on two consecutive days. After a rest period of 1 day TBI was applied, using an 8 or 10 MV linear accelerator, in one fraction (n = 50) or two fractions on two consecutive days (n = 43). Patients were irradiated anteriorly and posteriorly. TBI dose and schedules used were changed a few times since 1982 and are listed in Table 2. The dose rate was about 9 Gy/h.

GVHD

Prophylaxis for GVHD consisted of cyclosporin 3 mg/kg/day, given intravenously from 1 day before BMT until 3 weeks after BMT. Subsequently, cyclosporin medication was applied orally for a period of 3 months after BMT. Bone marrow grafts in allogeneic BMT were T-cell depleted to diminish severity of GVHD. Patients who were transplanted with marrow from a matched unrelated donor (MUD) (n = 3) received additional prophylaxis with antithymocyte globulin (ATG), 4 mg/kg/day intravenously for 5 days.

From 51 allogeneic transplanted patients, 28 developed GVHD. Of these, 10 patients had acute GVHD, 2 chronic and 16 patients had both acute and chronic GVHD. From the patients who developed acute GVHD, 9 had Grade I GVHD and 17 Grade II or more. When GVHD developed in Grade I, only local therapy was applied. When Grade II occurred prednisone was given 2 mg/kg/day orally, diminishing the dose after 10 days, if possible, to zero. When chronic GVHD developed prednisone was given orally, generally at doses of 20 mg to 40 mg/day.

Cataract Assessment

Follow-up was performed in the Departments of Hematology and Radiotherapy. Special attention was paid to cataract as a late side effect of radiotherapy. Examination of the eye lenses was performed at every visit at the Department of Radiotherapy and slides were made by fundus camera through dilated pupil until 1989. From 1989 examination by slitlamp was performed and slides were made at the Department of Ophthalmology, whether an opafication was seen or not. From 58 of the 93 patients also slides were made a few days before BMT. Follow-up period was calculated from BMT until the last ophthalmologic assessment. To distinguish between patients with differences in the severity of cataract, the classification system of Schipper (13,14) was used. The classification system was based on photographs of the posterior subcapsular radiation

cataract of the human lens, just as the nowadays accepted classification system (15). The system was refined to some extent, meaning that between every stage an intermediate stage was inserted. In Fig. 1 the cataract classification system is demonstrated.

Follow-up was set up in 1982 as control of the eye lenses every 12 months. Cataract-free interval was defined as the time interval between BMT and the date a cataract was assessed for the first time in a subclinical stage [Grade 0/I (n = 23), Grade I (n = 42), or Grade I/II (n = 5)]. Very few patients (n = 5) had a rapidly progressive cataract that developed and progressed to a relatively high stage within 1 year. These patients were included as well, but only if no sign of cataract was diagnosed at a previous examination. Nine patients were

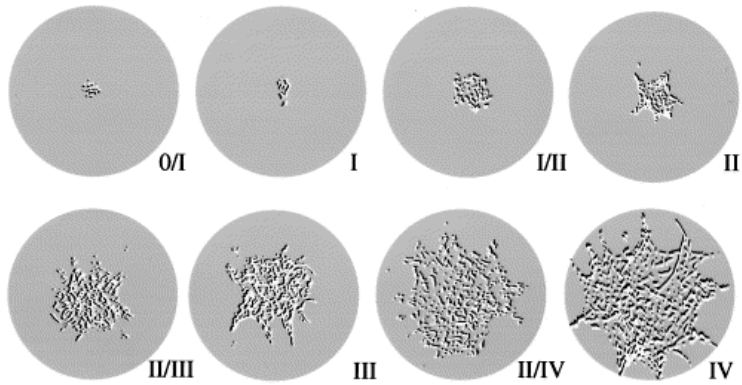

Fig. 1. Classification system for subcapsular posterior cataracts (13, 14). The numbers indicate the grade of the cataract Grade 0/I-II: low-grade cataract. Grade II/III-IV: symptomatic high-grade cataract.

excluded as they did not match with this definition, so 84 patients were analyzed (41 autologous and 43 allogeneic transplanted). Eight patients (7 autologous and 1 allogeneic transplanted) were considered censored, because they did not show a cataract at the end of the follow-up period. From the data of 20 patients who could be followed regularly for 40 months or more after the cataract was diagnosed in an early stage and before cataract surgery had to be performed, it was concluded that stabilization was obtained in all cases within 30 months. Therefore 30 months was assumed to be the progression duration of cataract, applying the present conditioning regimen for BMT.

According to this, the severity a cataract finally attained was defined as the grade in the classification system that occurred 30 months or longer after its detection When cataract surgery was performed within these 30 months, this was considered to be an endpoint and the cataract was regarded as stabilized. Following this definition, a group of 56 patients.

had a stable cataract. Two groups were analyzed according to the final severity. The first group consisted of patients with low-grade cataract: 0/I up to II (n = 25), the second group of patients with high-grade cataract: II/III, III, III/IV, and patients who underwent cataract surgery (n = 31)

Statistical evaluation

Kaplan-Meier product-limit estimates were used to evaluate the cataract-free duration. Patients who died from late relapse without having a cataract or who were lost to follow-up

because of other reasons without having a cataract or were cataract-free in May 1998 were considered censored. Differences between groups in the estimated cumulative cataract-free probabilities were examined using the log-rank test. Proportions in groups were compared using the chi-square test or the Fisher exact test. The Cox step-wise proportional hazards analysis was used to determine the independent contributions of prognostic factors thought to be influencing the cumulative cataract-free probability.
As only patients with a follow-up period of at least 23 months were included in the study, patients who died from toxicity of the treatment or of early relapse were not included and therefore did not have to be censored.

RESULTS

Of the 84 patients who were analyzed, 76 (89%) developed a cataract after a median follow-up period of 54 months (range 9-121). None of the 58 patients who had examination of the eyes before TBI had any sign of cataract. However, in the lenses of 30 of these patients (52%) vacuoles were seen. This finding reflected damage of the lens epithelium cells, probably caused by medication applied before BMT.

Effect of type of transplantation

Cataract-free interval: The probability of remaining free of cataract for at least 5 years, (Fig. 2) was 0.40 in the autologous and 0.18 in the allogeneic transplanted group. In recipients of autologous BMT, cataract was assessed after a median follow-up of 58 months versus 43 months in recipients of allogeneic BMT (Fig.2, Table 3). The difference in cataract-free interval (latency time) was significant ($p < 0.001$).

Severity of the cataract: In the 56 patients in whom the cataract was considered to be stable, severity of the cataract was not significantly different between autologous and allogeneic transplanted patients.Twelve (48%) autologous and 19 (61%) allogeneic transplanted patients had a high-grade cataract including those who underwent cataract surgery (Table 4). All other patients had a low-grade cataract.

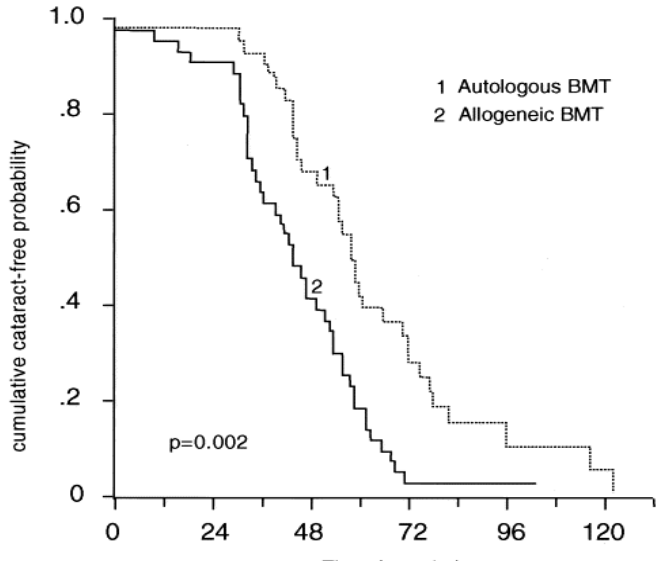

Fig. 2. Cumulative cataract-free probability of recipients of autologous and allogeneic BMT.

Table 3: Probability to remain cataract-free (84 patients)

	after 3 years		after 5 years		median time*	range	p†
	prob.	s.e.	prob.	s.e.			
Autologous	0.93	(0.04)	0.40	(0.08)	58	(30-121)	
Allogeneic	0.63	(0.07)	0.18	(0.05)	43	(9-102)	< 0.001
GVHD - (autol. and allog.)	0.92	(0.03)	0.33	(0.06)	55	(30-121)	
GVHD +	0.46	(0.10)	0.14	(0.07)	33	(9- 70)	< 0.001
Autologous	0.93	(0.04)	0.40	(0.08)	58	(30-121)	
Allog. GVHD -	0.84	(0.08)	0.16	(0.08)	46	(32-102)	< 0.001
Allog. GVHD +	0.46	(0.10)	0.13	(0.07)	33	(9- 70)	
Autol.	0.93	(0.04)	0.40	(0.08)	58	(30-121)	
Allog. Ster. ± or -	0.74	(0.08)	0.15	(0.07)	46	(15-102)	< 0.001
Allog. Ster. + +	0.44	(0.12)	0.12	(0.08)	33	(9- 70)	
Autol. 1 x 8 Gy	0.93	(0.04)	0.40	(0.08)	58	(30-121)	
Allog. 1 x 8 Gy	0.63	(0.17)	0.13	(0.12)	46	(9-102)	
Allog. 2 x 5 Gy	0.64	(0.15)	0.27	(0.13)	52	(18- 68)	= 0.001
Allog. 2 x 6 Gy	0.63	(0.10)	0.08	(0.06)	41	(30- 70)	

* Months after BMT † Log rank test
Abbrevations: Ster. ± or - = low dose or no steroids; Ster. ++ = intensive treatment with steroids; Autol.=Autologous; Allog.=Allogeneic.

Table 4: Final severity of cataract (in 56 patients with stable cataract)

	High grade*		Low grade†		p‡
Autologous	48%	(n=12)	52%	(n=13)	
Allogeneic	61%	(n=19)	39%	(n=12)	n. s.
GVHD – (autol. and allog.)	43%	(n=16)	57%	(n=21)	
GVHD +	79%	(n=15)	21%	(n=4)	0.013
Allog. GVHD –	33%	(n=4)	67%	(n=8)	
Allog. GVHD +	79%	(n=15)	21%	(n=4)	0.022
Allog. Ster. ± or –	35%	(n=6)	65%	(n=11)	
Allog. Ster. ++	93%	(n=13)	7%	(n=1)	0.002
Allog. 1 x 8 Gy and 2 x 5 Gy	33%	(n=4)	67%	(n=8)	
Allog. 2 x 6 Gy	79%	(n=15)	21%	(n=4)	0.022

* Grade II/III - IV and artificial lens† Grade 0/I – II‡ Fisher's exact test
Abbrevations: Ster. ± or – = no or low dose steroids; Ster. ++ = intensive treatment with steroids; Autol.= Autologous; Allog.= Allogeneic

Effect of GVHD

Cataract-free interval: The probability of remaining free of cataract for at least 5 years after BMT was 0.33 in all patients without GVHD (including autologous transplanted patients) (n = 62) and 0.14 in patients with GVHD (n = 22). The difference between these groups was statistically significant ($p < 0.001$). The cataract was diagnosed in patients without and with GVHD after a median follow-up of 55 and 33 months post-BMT, respectively, (Table 3).When autologous transplanted patients (n = 41), and allogeneic patients without GVHD (n = 21) and with acute and/or chronic GVHD (n = 22) were compared, a statistically significant difference was found with respect to the probability to remain free of cataract ($p < 0.001$) (Table 3). However, between allogeneic patients without and with GVHD the probability of remaining free of cataract was only different in the first 4 years after BMT. Median times to develop cataract in these patient groups were 58, 46 and 33 months, respectively (Table 3).

Severity of the cataract: High-grade cataract or cataract surgery was assessed in 43% of all patients without GVHD (16 of 37) and in 79% with GVHD (15 of 19). All other patients had a stable low-grade cataract ($p = 0.013$) (Table 4). When only allogeneic transplanted patients were analyzed for severity of cataract, the effect of GVHD was even more pronounced. High-grade cataract or cataract surgery occurred in only 33% of the patients without GVHD (4 of 12) and in 79% of the patients with GVHD (15 of 19) ($p = 0.022$) (Table 4).

Effect of steroid therapy

Cataract-free interval: Sixteen of the 43 allogeneic transplanted patients received steroid therapy for more than 12 months or received a dose of > 60 mg/kg/day for more than 2 months during the post-transplantation period. They were considered the “treated” group. The other 27 patients had no or short time and/or relatively low-dose prednisone. When autologous transplanted patients (n = 41) and allogeneic transplanted patients without (n = 27) and with (n = 16) intensive steroid treatment were compared with respect to the probability to stay free of cataract, a statistically significant difference was found (p < 0.001)(Fig. 3). Median times to develop a cataract were 58, 46 and 33 months, respectively (Table 3). The probability of remaining cataract-free differed between the two allogeneic transplanted groups until 4 years after BMT. Thereafter, just as in patients without and with GVHD, curves followed almost the same course (Fig. 3) (Table 3).

Severity of the cataract: High-grade cataract or cataract surgery occurred in only 35% (6 of 17) of the allogeneic transplanted patients treated with low-dose or no steroid therapy, versus 93% (13 of 14) of patients treated with steroids (p = 0.002) (Table 4).

Effect of TBI dose

Cataract-free interval: The probability of remaining cataract-free for at least 5 years after BMT for patients with autologous BMT (all conditioned with 1 x 8 Gy TBI) (n = 41) was 0.40, for patients with allogeneic BMT and conditioning with 1 x 8 Gy TBI (n = 8) 0.13 and for patients with allogeneic BMT conditioned with 2 x 5 Gy (n = 11) 0.27. For patients with allogeneic BMT and conditioning with 2 x 6 Gy (n = 24) the probability was 0.08 (Fig. 4) (Table 3). Median cataract-free interval was 58, 46, 52 and 41 months, respectively. The differences between these groups were statistically significant (p = 0.001) (Table 3).

Severity of cataract: As numbers were small and the biological effectiveness of 1 x 8 Gy and 2 x 5 Gy was almost comparable according to the linear quadratic model (31,32) the results of the allogeneic patients in these two regimens were analyzed together to determine the final severity of the cataract. High-grade cataract or cataract surgery occurred in 33% (4 of 12) of the patients conditioned with 1 x 8 Gy or 2 x 5 Gy and in 79% (15 of 19) of the patients conditioned with 2 x 6 Gy TBI (p = 0.022) (Table 4). All other patients had a stable low-grade cataract.

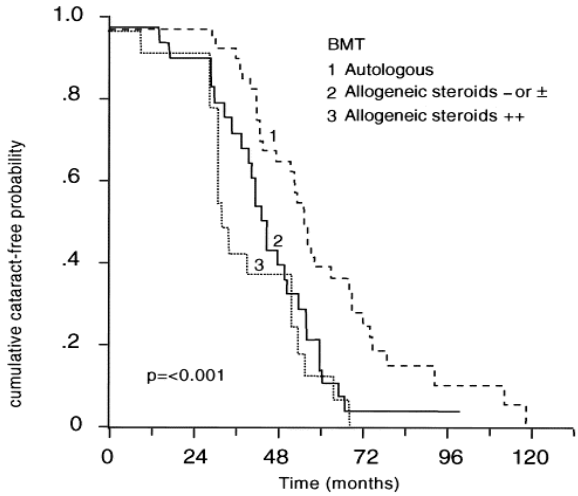

Fig. 3. Cumulative cataract-free probability of recipients of autologous and allogeneic BMT without and with intensive steroid treatment.

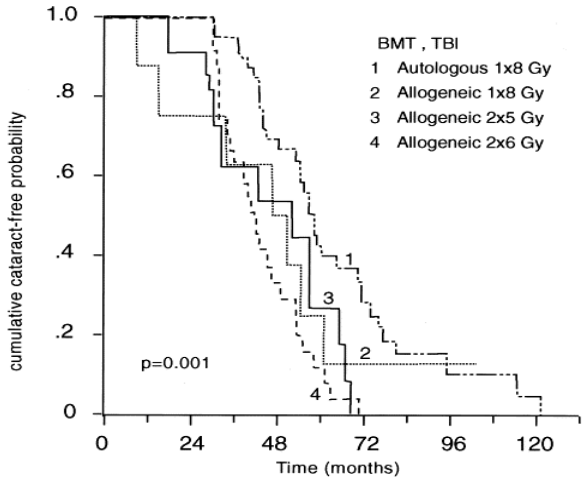

Fig. 4. Cumulative cataract-free probability of recipients of autologous and allogeneic BMT according to TBI scheme.

DISCUSSION

Ionizing radiation has since long been recognized as a cause of cataract formation (16,17). Also TBI, used as a part of the conditioning regimen for BMT, was described by several authors as a cause of cataract (5-17,18-20). TBI parameters reported to play a role were total dose, fractionation scheme, and dose rate (2,5-7,18,20-22). After conditioning for BMT with STBI , a cataract incidence of 80-100% is reported (18,19,21,23-25). Steroid therapy, often applied for GVHD post-transplantation, is also a known cause of cataract formation. It was observed to be cataractogenic in patients who had steroids for rheumatoid arthritis and after renal transplantation (26-29). After BMT, steroid therapy was reported to be cataractogenic in patients who had conditioning without TBI (5,26-29). In patients who had TBI as a part of their conditioning regimen steroids were reported to have an additive, synergistic effect on cataractogenesis (5,7,20,21,22,24). However, influences on the severity of cataract have only been investigated indirectly by counting retrospectively the patients who had cataract surgery; no data were reported about the presence of stable low-grade cataract causing no visual problems. In our series a high percentage of patients was expected to develop a cataract, as we used STBI or TBI in two fractions of 5 Gy or 6 Gy on two consecutive days. Although the latter schemes are fractionated, the 2 x 5 Gy regimen is radiobiological at least equally effective as TBI of one fraction of 8 Gy and the 2 x 6 Gy regimen is even more effective. Nevertheless, although it is obvious that the majority, if not all patients develop a cataract under those conditions, there appeared to be a large variation in latency time, progression rate, and final severity of the cataracts. Some lesions progressively opacified, whereas others remained small and caused no complaints.

In our present study we followed 93 patients who had TBI before BMT with respect to cataract formation and we routinely graded all cataracts according to a classification system. We classified the cataracts in a precise way to observe accurately what happens in the irradiated lens and to assess latency time, progression duration, and final severity of the cataract after stabilization. The classification system we used was introduced by Schipper, for classification of cataract in children irradiated for retinoblastoma (14). It was refined for the present study. The Schipper classification system is just like the nowadays accepted classification system for subcapsular posterior cataracts, based on photographs of posterior subcapsular cataracts with only a slight difference in number of grades (15).

In our series, 89% of the analyzed patients developed a cataract. As curves representing cataract-free interval do not show a plateau and as new cataracts were diagnosed even

120 months after BMT, probably in time all patients will develop a cataract. Nevertheless, although with the applied TBI schemes the total number of cataracts could not be influenced, it was shown that latency time and more important, final severity of cataract were varying largely dependent on the presence of GVHD and the treatment with steroids.

Cataract-free interval

Cataract-free interval or latency time was significantly different in recipients of autologous and allogeneic BMT during the whole follow-up period (Fig. 2) ($p<0.001$). Median cataract-free interval for patients who had autologous BMT was 58 months. For allogeneic transplanted patients without and with steroid treatment it was 46 and 33 months, respectively (table 3). However, after allogeneic BMT without and with steroid treatment latency time appeared to be only different in the first 4 years after BMT (Fig. 3) (Table 3). This was also true for allogeneic patients without and with GVHD. It most likely reflected the effect of steroid treatment for GVHD in the first years after BMT (Table 3).

Final severity

Stabilized low-grade cataract occurred in 52% of the patients who had an autologous BMT. In recipients of allogeneic BMT without steroid treatment, the incidence of stable low-grade cataract was 65%. However, of the allogeneic transplanted patients who had steroid treatment only 7% had a stable low-grade cataract and 93 % of the patients had high-grade cataract or cataract surgery (Table 4). Thus, the use of steroids had a striking effect, especially on the final severity of a cataract.

It appeared that autologous transplanted patients had a lower percentage of low-grade cataracts (52%) than allogeneic patients without steroid treatment for GVHD (65%). The reason for this is not completely clear, but might have been caused by pretransplant treatment, as recipients of autologous BMT suffered a high percentage of non-Hodgkin's lymphoma (NHL) and multiple myeloma (74% of all autologous transplanted patients). These patients are treated for a relatively long period with high dose steroids before the application of BMT.

Although all autologous transplanted patients were treated with the same TBI regimen and therefore all did not receive steroids for GVHD, there were marked differences in grading of the cataract. This may partly have been caused by pretransplant therapy but is probably also caused by individual variation in normal tissue reactions to radiotherapy. Also Zierhut et al. (30) mention the need for surgery, and therefore severe cataract, in 6 of 28 patients

who developed cataract after autologous transplantation. All of their patients also had the same (multifractionated) TBI regimen.

Influence of TBI dose and scheme

As different TBI doses and schemes were applied and dose and fractionation is reported to be a factor of influence on cataractogenesis (5,6,10,20,22), we also analyzed latency time and final grade of the cataracts for the three treatment groups in the study: 1 x 8 Gy, 2 x 5 Gy and 2 x 6 Gy. Median cataract-free interval after TBI was longest for recipients of autologous BMT conditioned with 1 x 8 Gy (58 months). For recipients of allogeneic BMT who were conditioned with 1 x 8 Gy (n = 8), 2 x 5 Gy (n = 11) or 2 x 6 Gy (n = 24) median cataract-free interval was 46, 52 and 41 months, respectively (Table 3). A stable high-grade cataract was diagnosed in 48% of the autologous transplanted patients, all conditioned with 1 x 8 Gy. In allogeneic patients it was 35% after conditioning with 1 x 8 Gy or 2 x 5 Gy, and 79% after 2 x 6 Gy conditioning therapy (Table 4). All other patients with a stable cataract had a low-grade cataract that caused little or no symptoms.

Although significant differences in latency time and final severity were found between the various treatment groups with univariate analysis, transplantation type, steroids, GVHD, and TBI dose were not considered independent prognostic factors by multivariate analysis. This can be explained by the observed strong association between these variables and the relatively small numbers per treatment group. The percentage of patients receiving steroid treatment in the 2 x 6 Gy group was 59% (19 of 32) versus 21% (4 of 19) in the 1 x 8 Gy and 2 x 5 Gy group. Therefore, although 2 x 6 Gy is radiobiologically more effective, the higher percentage of steroid treatment may have played a major role in cataract severity of the 2 x 6 Gy scheme.

To compare the biological effects of the schemes used, we calculated the biological effective dose (BED) with the linear quadratic model (31,32), considering the eye lens as a late responding tissue with an α/β = 3 Gy. The overall dose rate in our series was 9 Gy/h. The BED calculated for the lens for a scheme of 2 x 6 Gy was 33.8 Gy, for 2 x 5 Gy it was 25.2 Gy and for 1 x 8 Gy it was 26.7 Gy. Thus a scheme of 2 x 6 Gy appears to be certainly more effective than schemes of 2 x 5 Gy and 1 x 8 Gy, which are approximately comparable. In publications of cataract formation after TBI as a conditioning regimen for BMT, often a distinction is made only between single dose and fractionated TBI (6,20). The different fractionation schemes, however, can have a totally different biological effect depending on total dose, number of fractions and overall time (5-7,18-20). A fractionation scheme of 2 x 6 Gy, as we used, is biologically more effective than a single dose scheme

of 1 x 8 Gy and certainly than a highly fractionated scheme of 6 x 2 Gy or 12 x 1,2 Gy as used by Zierhut *et al*. (30) with BED values of 20 and 20, 16 Gy, respectively. In our opinion the biological effectiveness of a scheme should always be taken into account when comparing the effects of TBI, unless the BED values are approximately equal. Tichelli *et al*. (21) report on a scheme of 6 x 2 Gy over 3 days and Zierhut on a scheme of 12 x 1,2 Gy over 3 days. These schemes do indeed result in a lower cataract incidence, later occurrence of cataract, and less need for surgery (which roughly reflects severity) than STBI does. This must be mainly due to the lower BED of these fractionation schemes.

No relation between age and length of cataract-free interval or final grade of the cataract after TBI was found as in most studies (5,7,12,19). A few studies mention age older than 23-25 years as a risk factor (9,20); in our study relatively few patients were younger than 23 years. In the present study all subcapsular posterior cataracts were considered to be induced by the treatment, as this form of cataract is typical, both for radiation-induced cataracts and for steroid-induced cataracts, which are indistinguishable from each other (28,33). It is the least common type of cataract in the general population (17,18). Cranial irradiation was not taken into account because of the infrequency of this therapy (n = 2) in our study.

Until now no data were reported about stabilization or stable low-grade cataract and no attempts were made to correlate the incidence of the cataract with the BED.

CONCLUSION

This study showed by means of a regular, long-lasting follow-up and the use of a classification system, that latency time and final severity of a cataract after TBI for BMT vary strongly according to transplantation type, steroid treatment for GVHD, and probably biological effectiveness of the TBI scheme.

When no steroid therapy is applied, even after STBI the majority of patients will have a stable low-grade cataract, causing no or only minimal problems. Steroid treatment after BMT is a major cause of aggravation of visual impairment. Difference in severity of cataract between autologous BMT and allogeneic BMT without steroid treatment might be explained by steroid therapy used before autologous BMT.

In order to diminish high-grade cataract, other treatment approaches for GVHD seem mandatory. Furthermore, TBI parameters such as dose fractionation and total dose to the

eyes could be adapted in patients with low risk for central nervous system (CNS) relapse. Lowering the dose rate does not seem useful because of the long-lasting irradiation time. Dose fractionation in multiple fractions will probably prevent high-grade cataract in patients without steroid treatment and diminish the occurrence in other groups. Total dose on the eyes in STBI can be diminished simply by blocking of the eyes during irradiation of the anterior field. A relatively small dose reduction probably prevents high-grade cataract after STBI in patients without steroid treatment and diminishes it in other groups. Already some centers are applying this technique. It may, however, increase the risk for CNS-relapse and therefore the treatment results need to be analyzed. Other options are the use of heparin and verapamil, which might diminish the incidence of cataracts after BMT (20,34).

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Chapter 3

CATARACT AFTER TOTAL BODY IRRADIATION AND BONE MARROW TRANSPLANTATION: DEGREE OF VISUAL IMPAIRMENT

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Chapter 3

ABSTRACT

Purpose: To assess the degree of visual impairment as a result of cataract formation after total body irradiation (TBI) for bone marrow transplantation.

Material and Methods: The data of 93 patients who received TBI in 1 or 2 fractions as a part of their conditioning regimen for bone marrow transplantation were analyzed with respect to the degree of visual impairment as a result of cataract formation. The probability to develop severe visual impairment (SVI) was determined for all patients and the degree of visual impairment was assessed for 56 patients with stabilized cataract, using three categories: no, mild, or severe.

Results: For all 93 patients the probability of developing a cataract causing SVI was 0.44. For allogeneic patients, it was 0.33 without and 0.71 with steroid treatment ($p < 0.001$). All SVI-free probability curves reached a plateau distinct from the cataract-free curves. Apparently, cataracts developing late in the follow-up period rarely cause SVI. Of the patients with stabilized cataract, 32% had no visual impairment, 16% mild, and 52% severe impairment. No or mild visual impairment was present in 61% of all patients with stable cataract and no steroid treatment compared with only 13% of the patients treated with steroids ($p = 0.035$).

Conclusion: SVI occurs in only some of the patients (52%) with stable cataract after TBI for bone marrow transplantation in 1 or 2 fractions. Steroid treatment markedly increases the probability of developing visual problems as result of a cataract after TBI.

INTRODUCTION

Total body irradiation (TBI) is frequently used as part of the conditioning regimen for bone marrow transplantation (BMT) in a variety of hematological malignancies and non-malignant disorders such as aplastic anemia (1-4). Cataract formation as a result of ionizing radiation has since long been recognized (5,6). A conditioning regimen for BMT containing TBI, especially single-dose TBI (STBI) is known to be more cataractogenic than a regimen containing only chemotherapy (7-9). Treatment parameters, apart from TBI, such as steroid administration and prophylactic cranial irradiation, are also reported to influence cataract formation (7,9-11). In our center, STBI or TBI in 2 fractions is applied just as in most other centers for BMT in The Netherlands. The two schemes will probably cause a cataract in 80-100% of the treated patients (12-14). In a large part of the patients, however, cataract formation does not result in severe visual impairment (SVI).
Recently we reported a group of 93 patients treated at our center with STBI or TBI in 2 fractions (15). Ninety-one percent of the patients developed a cataract in varying grades of severity. Because it is not the cataract per se, but the visual impairment caused by the cataract that determines the quality of life, we decided to reevaluate the data of the 93 patients from a clinically more relevant point of view (i.e., the severity of visual impairment as a result of cataract formation after TBI before BMT).

METHODS AND MATERIALS

This analysis concerned 93 patients who had undergone TBI as part of their conditioning regimen for BMT (15). Forty-two patients had autologous and 51 allogeneic BMT. TBI was administered in one fraction of 8 Gy or 2 fractions of 5 or 6 Gy on 2 consecutive days, dose rate 9 Gy/h. The patient characteristics are listed in Table 1. The patients were followed annually with respect to cataract development and, consequently, their visual complaints as a result of the cataract. Follow-up was only ended after cataract surgery, which was always considered to be indicated by severe complaints as a result of high-grade cataract. The median follow-up period was 74 months (range 23-161).

Conditioning regimen, management of graft versus host disease (GVHD) and steroid treatment

All patients were conditioned with cyclophosphamide 60 mg/kg/d on 2 consecutive days

and TBI in 1 fraction of 8 Gy or 2 fractions of 5 or 6 Gy on consecutive days with an 8 or 10-MV linear accelerator. The overall dose rate was 9 Gy/hour throughout the entire study period. Graft vs host diease (GVHD) prophylaxis consisted of cyclosporin 3 mg/kg/d given i.v. for 3 weeks after BMT and subsequently p.o. for 3 months When a patient developed

Table 1. Characteristics of 93 patients

Characteristic		Result
Age (years)	Median	35
	Range	14 – 56
Gender	Male	38
	Female	55
Diagnosis	AML	18
	ALL	18
	CML	11
	NHL	32
	Multiple Myeloma	7
	Aplastic Anaemia	7
TBI dose	Autologous BMT	1 x 8 Gy (n = 42)
	Allogeneic BMT	1 x 8 Gy (n = 8)
		2 x 5 Gy (n = 11)
		2 x 6 Gy (n = 32)

Abbrevations: AML = acute myeloid leukemia; ALL = acute lymphoid leukemia; CML = chronic myeloid leukemia; NHL = non-Hodgkin's lymphoma; TBI = total body irradiation; BMT = bone marrow transplantation.

GVHD, local therapy was applied for grade I. For grade II GVHD, prednisone was given 2 mg/kg/d for 10 days, and then tapered if possible. For chronic GVHD, prednisone 20-60 mg/d p.o. was given. Patients who received steroid therapy for >1 year and/or ≥60 mg prednisone for 2 months were defined as the (intensively) treated group. All other patients were considered to be the not (or mildly) treated group.

Assessment of visual impairment

An examination of the eyes was performed annually between 1982 and 1994, and was always accompanied by an interview of the patients for complaints of visual impairment. When patients had visual complaints that could not be related to other ophthalmological problems and a cataract was found, these complaints were regarded as caused by the cataract.
Because the visual problems were caused in great part by glare, testing of the visual acuity could not be used to measure the visual impairment. We therefore decided to register the complaints as experienced by the patients into three categories: not present, mild or severe. *Mild* complaints were defined as slight visual impairment, noticed by the patient but not interfering with normal daily functioning and not considered as a reason for cataract surgery. Severe complaints were defined as visual impairment interfering with daily functioning or work and patients motivated to undergo cataract surgery when asked about it.
In our former study concerning the progression of the cataract after BMT, we found that progression never occurred >30 months after the cataract had been diagnosed (15). Thirty months were therefore assumed to be the progression duration, after which a cataract could be regarded as stabilized. Fifty-six of the 93 patients had a stable cataract with no, mild or severe visual impairment.
The degree of visual impairment as experienced by the patient was also compared with the grade of severity of the cataract according to the grading system we used in the former analysis.

Statistical analyses

The SVI-free survival was evaluated using Kaplan-Meier product limit estimates. Because SVI had the most important impact on quality of life, patients with no or only mild visual impairment were considered censored for this analysis.
Differences between groups in the estimated cumulative probability of being SVI-free were examined using the log-rank test.
Pearson's chi-square test (exact version) was used to compare proportions between groups. The Kruskal-Wallis test was used to relate the grade of cataract to the degree of visual impairment.

Chapter 3

RESULTS

Rate of visual problems after long-term follow-up

The overall incidence of cataract in the 93 patients was 91% as reported before (15). The overall incidence of SVI (visual impairment interfering with daily activities or work) at the last follow-up visit was 33%.

Stable cataract, without visual impairment

Eight patients did not develop cataract during the follow-up period. In the remaining 85 patients, 69 had no visual impairment the first time a cataract was observed, 8 had mild visual impairment, and 7 patients already had SVI when the cataract was observed for the first time. In 18 of the patients with cataract and no visual impairment, the absence of complaints remained unchanged for ≥ 30 months. They were therefore considered to have a stable cataract without visual impairment. All other patients experienced progression of their complaints, or could not be followed until the cataract was considered to be stable. When the characteristics of the 18 patients with stable cataract and no visual impairment were analyzed, 50% proved to be recipients of autologous and 50% of allogeneic BMT. Only 1 patient had been treated with steroids (Table 2).

Table 2. Visual impairment as a result of stable cataract

	BMT		Steroids		
Visual impairment	Autologous	Allogeneic	No or low-dose	Intensive	Total
No	9 (50%)	9 (50%)	17 (94.4%)	1 (5,6%)	18
Mild	5 (55.6%)	4 (45.4%)	8 (88.9%)	1 (11.1%)	9
Severe	10 (34.5%)	19 (65.5%)	16 (55.2%)	13 (44.8%)	29
Total	24	32	41	15	56
p	n.s.		0.035		

Abbrevations: n.s. = not significant; Autol. = Autologous; Allog. = Allogeneic; Ster.- or ± = no or low dose steroid treatment; Ster.++ = intensive treatment with steroids

Stable cataract with mild visual impairment

In 9 of the patients with mild visual impairment, the complaints remained unchanged for ≥ 30 months after the first diagnosis of the cataract. They were therefore considered to have a stable cataract with mild visual impairment. Of these 9 patients, 4 received allogeneic and 5 autologous BMT. Only 1 patient had received steroid treatment for GVHD (Table 2). None of the 9 patients needed cataract surgery.

Stable cataract with SVI

Twenty-nine patients developed cataract with SVI during their follow-up period. Of these 29 patients, 24 underwent cataract surgery after a median period of 65 months (range 30-180) after BMT. An analysis of the 29 patients showed that 65.5% (n = 19) received allogeneic and 34.5% (n = 10) autologous BMT (Table 2). Of the patients treated with steroids, a relatively high percentage developed a stable cataract with SVI (86,6%, 13 of 15) compared with patients not treated with steroids (39%, 16 of 41) (p = 0.035).

Cumulative probability of developing SVI

We determined the visual impairment-free follow-up period. This period was defined as the time interval between TBI and the onset of SVI. The 3- and 5-years SVI-free probability for all 93 patients is listed in Table 3. After 96 months, the probability to develop SVI reached a plateau, whith 56% of the patients free of SVI. Although follow-up was performed until 161 months after BMT, no new cases of SVI were detected (Fig. 1A).
Earlier, we reported on the cumulative cataract-free probability (interval between BMT and first appearance of cataract) (15). The results of the present study indicate that the probability of remaining without SVI stabilized (Fig. 1A), and the probability of remaining cataract-free continued to decrease (Fig 1B)
.

Transplantation type

A statistically significant difference in the SVI-free period was observed between autologous (n = 42) and allogeneic (n = 51) transplanted patients (p = 0.046) (Table 3). This probably was an effect of steroid treatment for GVHD, because the 5-year SVI-free

probability and SVI-free probability at stabilization did not differ for autologous patients and allogeneic patients not treated with steroids

Table 3. Probability to remain SVI-free

	After 3 y		After 5 y		Plateau phase			
BMT	Prob.	SE	Prob.	SE	Prob.	SE	Interval after BMT(mo)	p
All patients, n=93	0.98	0.02	0.80	0.04	0.56	0.06	96	-
Autologous, n=42	1	-	0.89	0.05	0.63	0.09	96	
Allogeneic, n=51	0.96	0.03	0.72	0.07	0.50	0.08	82	0.046
Autologous, n=42	1	-	0.89	0.05	0.63	0.09	96	
Allogeneic ster. ± or – n=30	1	-	0.89	0.06	0.67	0.11	82	<0.001
Allogeneic ster. ++ n=21	0.90	0.07	0.50	0.11	0.29	0.11	67	

Abbrevations : SE = standard error ; BMT = bone marrow transplantation ; ± or - = Low-dose or no steroids; ++ = intensive treatment with steroids.

Influence of steroid treatment

Allogeneic grafted patients not treated with steroids had a higher SVI-free probability compared to allogeneic patients who were treated with steroids (Fig. 1C). The cumulative probability of remaining free of SVI for at least 3 and 5 years, for autologous grafted patients (n = 42) and untreated (n = 30) and treated (n = 21) allogeneic patients, is listed in Table 3. The SVI-free period stabilized in the allogeneic group without and with steroid treatment 82 months (67% free of SVI) and 67 months (29% free of SVI), respectively, after BMT (p <0.001) (Fig 1C). Comparing the cataract-free probability for patients without and with steroid treatment and the probability of remaining without SVI, a plateau was again present in the curves representing the probability of remaining free of SVI (Fig 1C) that was absent in the cataract-free probability curves (Fig 1D).

In our former analysis, GVHD and TBI dose proved to interrelate closely with the use of steroid treatment after BMT (15). We therefore did not analyze their influence on SVI-free probability.

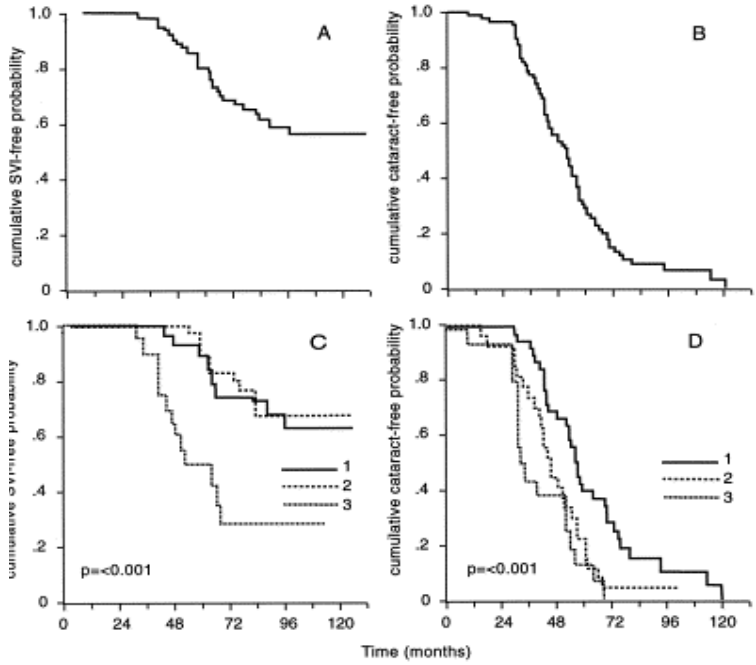

Fig. 1. (A) Cumulative SVI-free probability and (B) cumulative cataract-free probability of all patients in the study, and (C) cumulative SVI-free and (D) cumulative cataract-free probability of autologous and allogeneic grafted patients with and without steroid treatment. 1 = autologous BMT; 2 = allogeneic BMT not treated with steroids; 3 = allogeneic BMT treated with steroids.

Correlation of degree of visual impairment and grade of cataract

We compared the grade of visual impairment with the grade of severity of the cataract, as determined in the former analysis, in the same patient at the last follow-up visit. The mean grade of cataract proved to be significantly related to the degree of visual impairment ($p < 0.001$, Kruskal-Wallis Test).

DISCUSSION

Cataract formation is a known complication of TBI applied as part of the conditioning regimen for BMT (2,8-10,12,14,). Especially after STBI, a high cataract incidence has been reported. After TBI in multiple fractions, the cataract incidence has been found to be lower and to occur after a longer latency period (9,10,12-14,16-19). The rate of visual

impairment resulting from a cataract after TBI, however, appears to vary strongly. In a previous analysis, we described the cataract-free period and the severity of cataract occurring in 93 patients treated with TBI for BMT (15).

Because visual impairment is an important clinical endpoint, we reevaluated the data of these 93 patients for the degree of visual impairment as experienced by the patients.

The incidence of cataract found in the former analysis was high (91%), but analyzing the probability of all patients to develop SVI, we found only 44% had SVI at 96 months.

However, from the patients with stable cataract who were treated with steroids for GVHD, only a few (13%, 2 of 15 patients) had no or mild visual impairment. The probability of remaining without SVI for patients treated with steroids was only 28% at 67 months after TBI. Thus, patients with no or mild complaints as a result of cataract were predominantly found among patients who had no steroid treatment.

To measure the visual impairment we could not use the visual acuity, because the complaints were largely caused by glare, a visual problem occurring in bright light or focusing a light source in the dark, and not adequately quantifiable. This was the reason we graded the visual impairment as indicated subjectively by the patients as no, mild, or severe.

We also investigated whether the degree of visual impairment as indicated by the patients was related to the grade of severity of their cataract using the grading system we used in the former analysis (15). This was performed because we had the impression that patients' occupations played a role in the way they judged their visual problems, patients seemed to get used to a long-existing, stable cataract, and not all cataracts were located precisely in the center of the lens. However, a significant association ($p < 0.001$) was found.

Table 4. Visual impairment as a result of stable cataract in patients with no, mild or intensive treatment with steroids.

Visual impairment	Autologous BMT	Allogeneic BMT		Total
		Steroids ± or -	Steroids ++	
No or mild	14 (51.9)	11 (40.7)	2 (7.4)	27
Severe	10 (34.5)	6 (20.7)	13 (44.8)	29
Total	24 (42.9)	17 (30.3)	15 (26.8)	56

Significance: $p = 0.006$.
Abbrevations as in Table 3.
Numbers in parentheses are percentages.

Most authors only roughly estimated visual impairment by retrospectively determining the number of patients who underwent cataract surgery. We found a large number of patients with a stable cataract but no or only slight complaints. We also found that the probability of remaining free of SVI reaches a plateau distinct from the probability of remaining free of cataract, which continues to decrease. Cataracts developing late in the follow-up period probably do not give rise to SVI. This seems to be an important issue, because after TBI applied in multiple fractions cataracts tend to develop relatively late (10,17).

Analyzing the effect of steroid treatment on the SVI-free period, it appeared that steroid treatment shortens the interval to developing SVI and also decreases the SVI-free probability (29%) (Fig. 1C) (Table 3). After stabilization of the cataracts, the great majority of the allogeneic transplanted patients treated with steroids had SVI (85.6%, 13 out of 15) in contrast to autologous transplanted patients (41.6%, 10 out of 24) and allogeneic grafted patients not treated with steroids (35%, 6 out of 17 ($p = 0.006$) (Table 4).

The effect of GVHD on visual impairment is strongly related to steroid therapy. We therefore did not separately analyze the effect of GHVD. The analysis of the effect of the various TBI schemes we used was hampered because in some schemes patients more often had GVHD and steroid treatment than in others. This finding also emerged from the former analysis (15).

Steroid therapy is known to have an additive effect on cataractogenesis after TBI for BMT (7,9,13,16,17,21). An effect on severity of visual impairment after BMT was described as well, as reflected by an increased need for cataract surgery after steroid treatment, not only after STBI, but also after TBI in multiple fractions (10,17). When no steroids are given and no other risk factors such as cranial irradiation before BMT are present, the effect of TBI on the eye lens is mainly due to the applied TBI scheme, dose rate, total dose, and individual susceptibility for irradiation (9,10-14,16,17). Merriam and Focht (6) estimated the minimal cataractogenic dose for man 200 cGy. They also described the relationship between total dose and divided dose on cataractogenesis (22) Regarding TBI fractionation, the beneficial effect of applying TBI in multiple fractions was reported by many authors (9,10,12-14,16-19). Tichelli *et al*. found a 100% cataract incidence at 3,5 years after STBI and only 29% at 3,5 years after fractionated TBI, and the probability to develop cataract 6 years after fractionated TBI was 83%. However, cataracts developed later when TBI was fractionated and the need for surgery, which reflected the severity of visual impairment, was far less than after STBI (17). Belkacémi *et al.* (16) found in a large retrospective study of 1063 patients from the European Group for Bone Marrow Transplantation registry, a 60% cataract incidence after STBI, 43% after TBI of ≤ 6 fractions, and 7% after TBI > 6 fractions. They also found an effect of the dose rate; the cataract incidence and need for surgery were significantly lower at

low dose rates (16). Fife *et al.* (11) also reported an effect of the dose rate on the need for cataract surgery, reflecting the severity of visual impairment. The results, however, were never completely comparable because the fractionation schemes, dose rates, and total doses used vary for every center. In our opinion, the best way to compare the effects of the different TBI regimens is to calculate the biologic effective dose (BED) of a TBI regimen with the linear quadratic model, which takes into account total dose, dose rate, number of fractions, and overall time (23). We also mentioned this in our former report (15). Because steroid treatment is frequently needed in patients who underwent an allogeneic transplantation, the main factor able to decrease the incidence of a cataract formation with SVI is probably is the BED of the TBI regimen on the eye lens as determined with the linear quadratic concept (22). The BED should be as low as possible. This can be reached by fractionating the TBI, lowering the dose rate and/or lowering the total dose on the eye lens by shielding. The effect on cataractogenesis and the risk of relapse with shielding of the eye should, however, be investigated before it can be recommended safely on a routine basis.

In conclusion, the results of this study show that a relatively large number of patients treated with TBI in 1 or 2 fractions before BMT, will develop a cataract without SVI. Steroids strongly increase the probability of developing a cataract with severe visual problems. Cataracts developing late after BMT do not seem to give rise to SVI.

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Chapter 4

EYE SHIELDING DURING TOTAL BODY IRRADIATION FOR BONE MARROW TRANSPLANTATION IN CHILDREN TRANSPLANTED FOR A HEMATOLOGICAL DISORDER: RISKS AND BENEFITS

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Chapter 4

Summary

This is a retrospective analysis on 188 children who underwent total body irradiation (TBI) in one or two fractions before bone marrow transplantation (BMT) for a hematological disorder. While 139 children had eye shielding during TBI to decrease cataract formation, 49 did not. The blocks used for shielding caused cylindrical area of decreased dose intensity in the brain. The aim of the study was to determine if there was an increased risk of relapse in the eyes or in the CNS after shielding of the eyes. The probability and severity of cataract formation with and without shielding were also evaluated. None of the 49 children without shielding had a relapse in their eyes or in the CNS after BMT. Of the children with shielding, none had a relapse in the eyes two of the 139 (1.4%) had a CNS relapse. The incidence of cataracts without shielding was 90% (19 of 21 evaluable patients), while with shielding it was 31% (20 of 64). Severe cataracts were present in eight of 21 (38%) patients without and two of 64 (3%) patients with shielding. The probability of staying cataract free for at least five years was 0.77 with and 0.33 without shielding, at 8 years it was 0.53 and 0.24 respectively. The relative risk of developing a cataract without shielding *vs*. shielding was three (95% CI = 1.5; 5.9).

It appears that the incidence of relapse in the eyes and CNS is not increased when the eyes are shielded during TBI. Shielding increased the latency time of cataract formation and decreased the severity of cataracts.

INTRODUCTION

Total body irradiation (TBI) is an important part of the conditioning for bone marrow transplantation (BMT) for many hematological disorders in children and adults (1-3). Cataract is a known side effect of TBI (4-7). After single-dose TBI the probability of developing a cataract is reported to be 70-100% (6-12). In more than 50% of these patients visual impairment is severe and cataract surgery may be necessary (6,9). An effective way of decreasing the risk of severe cataracts is to lower the radiation dose on the eye lenses by shielding the eyes. We applied this technique successfully to children who had TBI before BMT in Leiden and Utrecht. Shielding of the eyes, however, is controversial as the eyes (just as the CNS) are considered to be extra-medullary sanctuaries (13). We evaluated the data of all children who had a BMT with TBI as part of the conditioning in Leiden and Utrecht with the aim of investigating relapse incidence in the eyes after eye shielding during TBI. As the blocks used for shielding, cause tiny but tall cylindrical areas of decreased dose intensity to the brain, we also evaluated the incidence of relapse in the CNS. Furthermore, we evaluated the effect of eye shielding on cataract formation.

METHODS

Study design

We evaluated retrospectively the risk of relapse in the eyes and CNS after shielding of the eyes during TBI. Participants included all children who had TBI as part of the conditioning regimen for BMT from 1980 until 1999, in the department of Pediatric Hematology of the University Medical Centers of Leiden and Utrecht in the Netherlands. As a second end-point, we also determined the incidence and severity of cataract formation and the probability of remaining cataract-free with and without eye shielding.

Patients

A total of 188 children underwent allogeneic (n = 181) or autologous (n = 7) BMT with TBI

as part of their conditioning regimen. All children were transplanted for a hematological disorder (Table 1). Before BMT, the majority of the patients was treated for their disease with systemic chemotherapy and elective CNS therapy following the appropriate protocols of the Dutch Childhood Leukemia Study Group. Within the framework of these protocols, 18 of the

Table 1 Patient characteristics

		No eye shielding	Eye shielding	Total
Age (years)	Mean	8.7 (range 0.85-16.3), s.d. 4.7	9.2 (range 1.7-17.2 s.d. 4.0	9.1 (range 0.85-17.2), s.d. 4.2
Gender	Male	25	83	108
	Female	24	56	80
Diagnosis	ALL	24	68	92
	AML	15	44	59
	CML	2	6	8
	MDS	6	18	24
	NHL	2	3	5
Type of BMT	Autologous	2	5	7
	Allogeneic	46	125	181
Prev. Cranial irradiation	24 Gy	8[a]	2	10
	18 Gy	2	2	4
	<18 Gy	2	2	4

[a]Of these eight patients, three patients who had therapeutical cranial irradiation of 24 Gy also had prophylactical cranial irradiation before BMT of <18 Gy. Prev. = previous.

Table 2 TBI characteristics

Age (years)	Number of fractions and dose[a] per fraction (Gy)	No eye shielding	Eye shielding	Total
<2	1 x 5	4	0	4
	1 x 6	1	1	2
2 - 4	1 x 7	1	16	17
4 - 9	1 x 7.5	21	64	85
≥10 before 1990	1 x 8	20	10	30
≥10 after 1990	2 x 6	1	49	50

[a]Dose rate instantaneous 25 – 27 cGy/min; mean 15 – 18 cGy/min.

Children received irradiation to the CNS before BMT as prophylaxis (n = 8) or therapy because of CNS involvement at diagnosis or relapse (n = 7) or both (n = 3). The conditioning regimens for BMT in Leiden and Utrecht were identical. Conditioning always consisted of chemotherapy plus TBI. In 139 of the 188 children, transplanted after 1988, shielding of the eyes was applied during TBI; in 49 children the eyes were not shielded. Table 1 shows patient characteristics (age, gender, diagnosis, type of BMT, previous cranial irradiation, and eye or CNS involvement before BMT). Table 2 gives the characteristics of the TBI (total dose, dose rate, fraction size and age in relation to TBI).

Table 3 Involvement of eyes or CNS before and after BMT

Disease	At diagnosis				At relapse before BMT				At relapse after BMT			
	Eye	CNS	BM + CNS	BM + eye	Eye	CNS	BM + CNS	BM + eye	Eye	CNS	BM + CNS	BM + eye
AML (n = 92)	—	—	2	—	1	2	—		—	—	1	—
ALL (n = 59)	—	—	4	2	—([a]1)	13([b]1)	3		—	1	—	—
NHL (n = 5)	—	—	—	—	—	—	—		—	—	—	—
MDS (n = 24)	—	—	—	—	—	—	—		—	—	—	—
CML (n = 8)	—	—	—	—	—	—	—		—	—	—	—
Total	—	—	6	2	1	15	3		—	1	1	—

[a]one patient relapsed in the CNS at first relapse and in the eye at second relapse.
[b]one of tyhe 13 patients relapsed in the CNS at first relapse and again at second relapse.

Table 3 gives characteristics of the disease with respect to eye or CNS involvement (infiltration of the eyes or CNS at primary diagnosis, number and place of CNS/eye relapses) before BMT.

Preparative regimen and GVHD prevention

Besides use of TBI, the preparative regimen for AML, CML and MDS consisted either of cyclophosphamide alone (60 mg/kg i.v.) on two consecutive days (n = 38), or the combination of cyclophosphamide plus Ara-C (2000 mg/m^2/day i.v.) on two consecutive days (n = 48). For patients suffering from ALL or non-Hodgkin's lymphoma (NHL) the conditioning consisted either of cyclophosphamide alone (n = 40) or of cyclophosphamide

plus VP16 (350 mg/kg/day i.v.) on two consecutive days (n = 51). In total, 11 children (ALL = 6, AML = 3, CML = 1 and MDS = 1) had TBI in combination with other chemotherapy. TBI was applied after 1-day rest and given in one single fraction, or in two fractions on two consecutive days (Table 2). Six children, aged less than 2 years and grafted before 1990, were treated with a single fraction of 5 Gy (MDS = 1 and AML = 3) or 6 Gy TBI (ALL = 2). After 1990 no TBI was applied when children were younger than 2 years old. GVHD prophylaxis consisted of cyclosporin-A ± methotrexate. No T-cell depletion of the bone marrow was performed, except for patients who were recipients of a matched unrelated donor graft. When patients developed GVHD methylprednisolone was given (2 mg/kg/day) i.v. until the disease subsided. Subsequently it was continued orally, tapering the dose to zero if possible. When GVHD was progressive the dose of (methyl)prednisolone was raised temporarily and sometimes combined with other forms of immune-suppressive therapy. Prednisolone therapy was given to 34 patients for acute or chronic GVHD; 25 of these 34 patients were treated with steroids for 3 months or more after BMT. The relatively small number of children that needed long-term steroids for GVHD was caused by the fact that in Leiden children received complete intestinal decontamination in strict protective isolation for about 30 days post-BMT, resulting in a reduced number of patients suffering from GVHD.

TBI

TBI was given with linear accelerators. In Leiden, a 4 or 6 MV linear accelerator was used, while in Utrecht, an 8 or 10 MV accelerator. Instantaneous dose rates were 25 cGy/min (Leiden) and 27 cGy/min (Utrecht), overall dose rates being 18 and 15 cGy/min, respectively, throughout the whole study period. TBI doses and the number of fractions varied, according to the age of the child (Table 2). During the study period, the fractionation regimen for children older than 10 years was changed from one to two fractions. In both Leiden and Utrecht patients were irradiated in a lying position anteriorly and posteriorly. When eye protections were applied, they consisted of ∅ 3 cm leaden blocks (Leiden) or ∅ 3 cm stainless-steel cylinders (Utrecht) placed in the anterior field only. The dose reduction achieved by blocking of the eyes was 55% of the total TBI dose, calculated midplane at the level of the umbilicus of the patient in Leiden and 58% of the total TBI dose in Utrecht.

Assessment of eye- and CNS involvement and cataract assessment

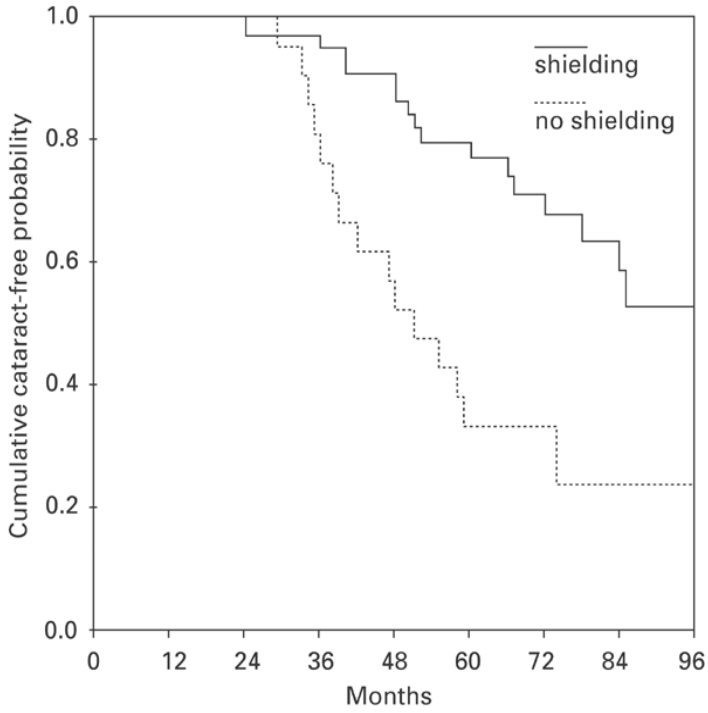
CNS involvement was defined as the presence of blast cells in the cerebrospinal fluid or the presence of cranial nerve palsies. Lumbar puncture was performed routinely at diagnosis, when a systemic relapse was diagnosed, or because of a clinically suspected CNS relapse. Eye involvement was determined by an ophthalmologist by physical examination of the eye (fundoscopy), combined, if indicated, with echography, CT-scanning and aspiration cytology when possible. Sometimes eye localizations were diagnosed on clinical appearance at fundoscopy (14). Fundoscopy was only performed when CNS involvement was diagnosed or when eye involvement was suspected. Eight patients showed involvement of the CNS (n = 6) or eyes (n = 2) at diagnosis. In all, 18 patients experienced a relapse in the CNS as the site of first relapse prior to BMT and only one patient had a relapse in an eye as the site of first relapse. Of the 18 patients with a CNS relapse, two had a second relapse before BMT (Table 3). Examination of the eyes for cataract development was performed during yearly routine split-lamp examination in the beginning of the follow-up period of each patient. In later years, the examination by split-lamp occurred in nearly all patients at more irregular intervals. To study the effect of eye shielding on cataract development, children were only included when they had at least one examination of the lenses, performed 2 years or more after BMT. The diagnosis “severe cataract” was used when corrective surgery was performed, or when the need for cataract surgery had been clearly established but surgery had not yet been performed.

Statistical evaluation

SPSS version 10.0 was used for statistical evaluation of the data. Kaplan-Meier product-limit estimates were used to evaluate cataract- free duration. For evaluation of the cataract-free duration only patients were included who had fundoscopy 2 years or more after BMT. Patients who died from a late relapse or other causes without having a cataract, who were lost from (ophthalmic) follow-up without having a cataract or were cataract-free on January 2001, were considered censored.

Table 4 Site of first relapse after BMT

Disease	Eye	CNS	BM	BM+CNS	Elsewhere	BM+elsewhere	Total
AML			19	1			20
ALL		1	29		3	3	36
NHL			1		1		2
CML			2				2
MDS			5		1		6
Total	0	1	56	1	5	3	66



F**igure 1.** Cumulative cataract-free probability of 85 children, without (n = 21) and with (n = 61) eye shielding during TBI (p = 0.001), who had an ophthalmic follow-up of ≥ 2 years after BMT.
Of the 188 patients who had a BMT, 66 experienced a relapse of their disease after BMT. Only the first relapse is considered. The site of the first relapse after BMT is shown in

RESULTS

Table 4. Of the 49 patients who had no eye shielding during TBI, none had a relapse in their eyes or the CNS. From the 139 patients who had eye shielding none had a relapse in the eyes while two had a CNS relapse; one a solitary CNS relapse and the other a combined bone marrow and CNS relapse. The patient who had a solitary CNS relapse suffered from ALL and had also a CNS relapse before BMT. The patient with a combined bone marrow and CNS relapse suffered from AML and had no relapse before BMT and no CNS involvement at diagnosis

A total of 129 children survived for 2 years or more after BMT. They were therefore eligible for cataract assessment. Of these children, 44 were not evaluable as they had had no ophthalmic follow-up 2 years or more after BMT. In all, 85 children were evaluable of whom 64 had eye shielding during TBI and 21 had none. All but two of the 21 children without shielding of the eyes, developed a cataract (90%). In six of these 19 cases cataract surgery was performed and, in two cases, cataract surgery was judged to be necessary, but had not yet performed, giving an incidence of 38% severe cataract in patients without eye shielding. Of the 64 children who had shielding, 44 had no cataract at the last follow-up visit. In 20 patients a cataract developed and two underwent cataract surgery, indicating severe cataract in only 3% of the 64 children (follow-up 24–118 months). The probability of remaining cataract-free was 0.33 (s.e. = 0.10) at 5 years follow-up for the children without lens shielding, while at 8 years follow-up it was 0.24 (s.e. = 0.09). For children with shielding of the eyes during the TBI the probability of remaining cataract-free was 0.77 (s.e. = 0.06) at 5 years and 0.53 (s.e. = 0.10) at 8 years after BMT (Figure 1). Longer follow-up data, concerning the probability of remaining cataract-free, cannot be given, as ophthalmic follow-up was irregular after 96 month.

The influence of previous cranial irradiation on the incidence and severity of cataract formation without and with shielding could not be assessed, as nine of the children who had cranial irradiation prior to BMT (n = 18) did not survive for 2 years (n = 7) or did not have an examination of the eyes 2 years or more after BMT (n = 2).

The influence of steroid therapy for GVHD (n = 34) on the probability of staying cataract-free was assessed for the children without and with eye shielding. However, the number of evaluable patients who were treated with steroids (24 of the 34 patients, of whom only 19 were treated with steroids for 3 months or more) appeared to be too small to draw any conclusions.

The relative risk (RR) of developing a cataract for patients without eye shielding was three times the risk of patients with shielding (95% CI 1.5; 5.9) (p = 0.002); when patients treated with steroid therapy were excluded, the RR for patients without shielding was 2.9 (95% CI 1.4; 5.9) (p = 0.003).

DISCUSSION

When TBI is used as part of the conditioning regimen for BMT, and especially single-dose TBI, severe cataract needing cataract surgery is observed in a high percentage of the patients (6,7,9). This was also confirmed in the present study. Total dose, fractionation regimen and dose rate of the TBI are important factors influencing cataract formation and its severity (2,6,10,12,15,16,17). Steroid treatment is known to induce cataracts in its own right (18-20) and can play an additional and synergistic role in the cataract formation after BMT (6-8,12,16). Age and cranial irradiation are mentioned as well by some authors as factors that might influence the formation of cataract (12,21).
In our centers, lens shielding during TBI was used in children to decrease the incidence and severity of cataract after BMT. By lens shielding, using the technique as done in most centers (irradiation anteriorly and posteriorly) and applying shielding in the anterior field, the dose on the eye lens can be lowered to about 55% - 60% of the total TBI dose. The theoretical drawback of eye shielding is, however, that by lowering the dose on the eyes, residual leukemic cells might be able to survive. This may happen not only in the eyes, but a part of the CNS also has to be regarded as at risk. The fact is that the blocks used for shielding in the anterior field cause tiny but tall areas of decreased dose intensity into the brain. Eye shielding, therefore, is controversial.

Influence of eye shielding on relapse rate in eyes and CNS

CNS relapses are reported to precede a systemic relapse and to worsen prognosis (13,22-25). Relapse in eyes and CNS after BMT, however, appeared to be rare compared to the incidence of relapse in eyes and CNS after conventional treatment without the use of BMT (26). Since cataract is a very disagreeable late complication in children and young adults, we decided to use eye shielding during TBI to prevent severe cataracts. In the present retrospective study, evaluating the data of all children treated until 1999, no relapses occurred in eyes or CNS of the children who had no eye shielding during the TBI (n = 49).

In the children who had lens shielding (n = 139) no relapses were observed in the eyes; in the CNS, a relapse occurred in two out of the 139 patients. The CNS relapse percentage after BMT and TBI with eye shielding can thus be considered as very low: 1.4% (1.7% when only patients with acute leukemia are considered). In the literature an incidence of CNS relapse after BMT of 3.3 – 8.7 % for ALL and of 0.2 – 1.4 % for AML is reported (in patients who had TBI without eye shielding) (27-30). Little is published about the incidence of CNS relapses after BMT in patients suffering from CML, NHL and MDS.
There are some reports in the literature about an increased risk in the CNS, after BMT in patients suffering from acute leukemia who had a previous history of CNS involvement (27,28). Singhal *et al*, (29) however, found no evidence of an increased risk of CNS involvement at presentation of the disease in 487 patients grafted for acute leukemia in first remission. In their series, only a few patients (n = 11) had CNS involvement at presentation. In our study, 27 (13 without and 14 with eye shielding) out of 188 patients had eye/CNS involvement at presentation or at relapse prior to BMT. Despite the lower irradiation dose to the eyes and part of the brain during the TBI, because of shielding in 139 of the patients, only two developed a relapse in the CNS. One of these two had CNS involvement at relapse before BMT. After BMT none of the patients had an ocular relapse. As patients with eye involvement represent usually only a very small percentage, there are few or no publications about the risk factors for developing a recurrence in the eyes after BMT. From our data, no definite conclusions can be drawn with respect to the probability of an increased risk of CNS or eye involvement after BMT in patients with CNS/eye involvement, because of the small number of relapses. It seems, however, to be the best policy not to shield the eyes of patients with a history of CNS or eye involvement prior to BMT.

Influence of eye shielding on cataract formation

A cataract developed in 90% of the children without lens shielding and 38% of them had a severe cataract. Of the children with shielding only 31% developed a cataract and 3% needed cataract surgery. The probability of staying cataract-free when eye shielding was used during TBI was significantly increased (Fig.1). The RR of patients without shielding the eyes compared to patients with shielding was three. No effect of steroid treatment could be found, because of the small number of evaluable patients who needed long- term steroid treatment for GVHD (n = 19). Cataracts appear to develop after a significantly longer latency time with shielding but eventually they appear in a considerable proportion

of patients. The major benefit from eye shielding, however, seems to be reduced severity of the cataracts as reflected by the reduced need for cataract surgery.

As cataract formation is described even after low radiation doses (15), it is probably impossible to avoid cataracts completely after TBI. Also, after fractionated TBI (multiple fractions), which similar to eye shielding reduces the biological effectiveness of the TBI on the lens, cataracts will still develop (6,10,12,16,17,31). However, when the biological effectiveness of the TBI is decreased, cataracts are known to develop after a longer latency period (16,6). In earlier studies (6,7) it was found that cataracts developing late in the follow-up period, progress slowly and stabilize at a lower grade, giving minimal visual impairment (9).

The fact that shielding is probably safe for children treated with single-dose TBI or TBI in two fractions does not necessarily mean that shielding can also be applied safely during fractionated TBI using multiple fractions. The biological effectiveness of fractionated TBI on the eye lens varies according to fraction size and total dose. Its effectiveness, however, on the leukemic cells varies too, although probably to a lesser extent (16). Shielding the eye lens during fractionated TBI could therefore theoretically have a different effect on the incidence of eye or CNS relapse compared to shielding during single-dose TBI.

CONCLUSION AND RECOMMENDATIONS

Shielding of the eye lens during TBI for BMT in one or two fractions, to prevent cataract formation or diminish its severity, does not seem to lead to an increased incidence of relapse in the eyes or the CNS after BMT. The probability of developing a severe cataract is markedly reduced after TBI with eye shielding.

Every patient of whom the eyes are planned to be shielded during TBI should have fundoscopy before starting the BMT procedure, to exclude disease infiltrates in the eyes. In patients with eye or CNS involvement prior to BMT no eye shielding should be used.

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Chapter 5

DOSE-EFFECT RELATIONSHIP FOR CATARACT INDUCTION AFTER SINGLE-DOSE TOTAL BODY IRRADIATION AND BONE MARROW TRANSPLANTATION FOR ACUTE LEUKEMIA

Int J Radiat Oncol Biol Phys. 2002; 52: 1367-1374

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Chapter 5

ABSTRACT

Purpose: To determine a dose-effect relationship for cataract induction, the tissue-specific parameter, α/β, and the rate of repair of sublethal damage, μ value, in the linear-quadratic formula have to be known. To obtain these parameters a large series of patients treated with different doses and dose rates is required. The data of patients with acute leukemia treated with single-dose total body irradiation (STBI) and bone marrow transplantation (BMT) collected by the European Group for Blood and Marrow Transplantation (EBMT) were analyzed.

Methods and Materials: Data of 495 patients who underwent BMT for acute leukemia, and had STBI were analyzed using the linear-quadratic concept. The end point was the incidence of cataract formation after BMT. Of the analyzed patients, 175 were registered as having cataracts. Biological effective doses (BEDs) for different sets of values for α/β and μ were calculated for each patient. With Cox regression analysis, using the overall chi-square test as parameter, evaluating the goodness of fit, α/β and μ values were found. Risk factors for cataract induction were BED of the applied TBI regimen, allogeneic BMT, steroid therapy for >14 weeks, and heparin administration. Patients who received steroid or heparin treatment were excluded, leaving only the BED as a risk factor. Next, the most likely set of α/β and μ values was obtained. With this set, cataract-free survival rates were calculated for specific BED intervals. Cataract incidences were obtained as function of the BED at 120 months after STBI.

Results: The use of BED instead of the TBI dose enabled the incidence of cataract to be predicted in a reasonably consistent way. With Cox regression analysis for all STBI data, a maximum chi-square value was obtained for $\alpha/\beta = 1.75$ Gy and $\mu = 0.75$ h^{-1}. When Cox regression analysis was applied for patients without steroid treatment, a maximum chi-square value was obtained for $\alpha/\beta = 1$ Gy and $\mu = 0.6$ h^{-1}. Repeating Cox regression using the data of patients who had no post transplant steroid treatment and also no heparin, we found $\alpha/\beta = 0.75$ Gy and $\mu = 0.65$ h^{-1}. Increased cataract incidence was observed after steroid treatment of >14 weeks and heparin administration.

Conclusion: The α/β value of 0.75 Gy and a μ value of 0.65 h^{-1} found for the eye lens are characteristic for late-responding tissues. The incidence of cataract formation can now be quantified, taking into account the values calculated for α/β and μ, TBI dose and dose rate. Also, reduction in cataract incidence as a result of lens dose reduction by eye shielding can be estimated.

INTRODUCTION

Bone marrow transplantation (BMT) has improved the long-term survival of patients treated for hematologic malignancies. Total body irradiation (TBI) is an important part of the conditioning regimen for BMT. Cataract formation is one of the widely reported late effects after TBI. Several factors are known to influence cataractogenesis after BMT, including TBI dose, fractionation scheme, radiation dose rate, steroid treatment (1-10), and the administration of heparin (7,8).

The data from a cohort of 2149 patients treated for acute leukemia, collected by the European Group for Blood and Marrow Transplantation (EBMT), were evaluated in an earlier analysis for risk factors by Belkacémi *et al.*, (7). In that study, the authors concluded that single-fraction TBI (STBI), high dose rate (≥0.04 Gy/min), age (>23 years), allogeneic BMT, and steroid administration (>100 days) were associated with an increased risk of cataract formation. Heparin administration was found to be a protective factor in the whole population and in the STBI subgroup. Cataract grading could not be performed because the study was retrospective and the participating centers did not use the same grading systems.

A dose-effect relationship for cataract formation, taking into account all parameters of the different TBI regimens, is presently not available. The linear-quadratic and biologic effective dose (LQ-BED) concept (11,12) has not been previously applied to quantify the influence of radiation parameters (total dose, number of fractions, dose per fraction, dose rate) on cataract formation. In this concept, the occurrence of a certain biologic effect (e.g., cataract incidence) depends on the dose in a linear-quadratic fashion. To calculate the incidence for a certain regimen, apart from the radiation parameters, two other parameters must be known: the tissue-specific parameters α/β and the rate of sublethal damage repair μ, (or the half-time for repair). For a number of early and late responding tissues α/β and μ have already been reported (13). For human lens tissue, these parameters are still unknown.

We applied the LQ-BED concept with different sets of α/β and μ values to the EBMT data set comprising STBI data. To search for the best combination of α/β and μ values, we used the Cox regression analysis to the BED sets. Furthermore, risk factors could be identified. After constructing a homogeneous group of patients and again after determination of the best combination of α/β and μ, a dose-effect relationship could be derived. Because α/β and μ are tissue-specific and independent of how radiation is

applied, the values of α/β and μ derived from STBI data also apply to fractionated radiotherapy.

The objective of this study was to determine the parameters α/β and μ for the eye lens to obtain a dose-effect relationship for cataract formation and to identify risk factors.

Table 1. Patient characteristics (n = 495)

Age (y)	Mean		25 ± 12	
	Range		1-57	
Gender	Male		297	(60)
	Female		198	(40
Type of disease	ALL		247	(50)
	AML		244	(49)
	AUL		4	(1)
Conditioning chemotherapy	Cy 120		371	(75)
	Cy 200		6	(1)
	Other drugs		117	(24)
BM type	Autologous		149	(30)
	Allogeneic (GISD)		346	(70)
Medical treatments	Steroids*	Administered	126	(25)
		No	325	(65)
		Unknown	44	(10)
	Heparin (VOD prevention)	Administered	30	(6)
		No	455	(92)
		Unknown	10	(2)
Radiotherapy parameters	TBI doses (Gy)		6 -11.82	
	Dose rates (Gy/h)		0.108 -18	

Abbreviations: ALL = acute lymphoblastic leukemia; AML = acute myeloblastic leukemia; AUL = acute undifferentiated leukemia; Cy = cyclophosphamide; Cy 120 = 60mg/kg, 2 consecutive days; Cy 200 = 50 mg/kg, 4 consecutive days; BM = bone marrow; GISD = genotypically identical sibling donor; TBI = total body irradiation; VOD = veno-occlusive disease.
*Steroids administered for any reason at a dose of 1 mg/kg/d.
Data presented as the number of patients, with the percentage in parentheses, unless otherwise noted.

METHODS AND MATERIALS

Patient characteristics

The analysis concerned data of 495 patients collected in the EMBT registry. The patients were treated for acute leukemia and underwent BMT in the first or second complete

remission. They received STBI as part of the conditioning regimen. In 1 case, the dose rate was not registered.

Cataract formation was diagnosed by slit-lamp examination performed routinely or because patients complained about visual impairment in 175 (35.4%) of the 494 evaluated patients. Surgery for severe cataract formation was required in 88 (50%) patients after a median period of 9 months after diagnosis of cataract formation. Patient characteristics are presented in Table 1.

LQ-BED concept

The occurrence of a biologic effect E depends on the dose in a linear and quadratic fashion:

$$E = n (\alpha d + \beta d^2) \quad (1)$$

where n is the number of fractions, d is the dose per fraction, and α, β are constants (11, 12). From that equation, the BED can be derived: $BED = nd [1+d/(\alpha/\beta)]$.

For single-dose irradiation using a low dose rate, $BED = RT [1 + kR/(\alpha/\beta)]$, where R is the dose rate, T is the treatment time, and $k = 2[1-\{1-\exp(-\mu T)\}/(\mu T)]/\mu$. The factor k depends on the sublethal damage repair rate μ during the low-dose rate irradiation, and treatment time T. The parameter μ is related to the half-time for repair of sublethal damage, where half-time = ln2/μ (14).

The strength of the LQ-BED concept is that for high and low-dose rate treatments, the BED values resulting in a specific isoeffect, in our case a certain percentage of cataract incidence, are equal.

The value of α/β is about 10 Gy for acute reacting tissues, and 1-4 Gy for late reacting tissues. The rate of sublethal damage repair μ is about 1 h^{-1} for acute reacting tissues and ranges between 0.3 and 0.9 h^{-1} for late reacting tissues (13). The human eye lens is considered to be a late responding tissue. For cataract induction, an α/β of 1.2 Gy (range 0.6-2.1) is the only value reported (13) from the responses of a murine lens model to fractionated irradiation (15). For the human eye lens, the α/β and μ values for cataract induction are unknown.

To derive a dose-effect relationship for cataract induction from LQ-BED concept application, the TBI parameters, cataract incidences at a specific time after TBI, and the

α/β and μ values have to be known. In this study, the STBI parameters and cataract incidence data were obtained from the EMBT registry.

For each STBI treatment, a specific BED was calculated using different data sets of α/β and μ values (e.g., α/β = 0.5 Gy with μ = 0.25 - 2 h^{-1}, α/β = 0.75 Gy with μ = 0.25 - 2 h^{-1}). A total of >40 combinations of α/β and μ values were applied to calculate the BED values for the 494 patients.

Statistical analysis

To determine the combination of α/β and μ associated with the highest overall chi-square, we applied Cox regression analysis to the BED values calculated with different sets of α/β and μ. Factors associated with an increased risk of cataract induction (apart from total dose and dose rate) and previously reported by Belkacémi *et al.* (7), such as transplant type, steroid therapy, age, and heparin administration were also reanalyzed using covariate analysis. The level of significance of *p*-value was set at $p = 0.05$.

Kaplan-Meier product-limit estimates were used to evaluate cataract-free survival rates for specific BED ranges. Cataract incidences were derived as a function of BED at 120 months after TBI. This point was chosen because of the plateau observed in cataract formation.

RESULTS

Parameters α/β and μ for the whole data set

With Cox regression analysis, the overall chi-square test was used as parameter to evaluate the goodness of fit for all the 494 STBI data. A maximal chi-square value was obtained with an α/β value of 1.75 Gy and a μ value of 0.75 h^{-1}.

Risk factors and α/β and μ values for patients not treated with steroids and heparin

The factors associated with an increased risk on cataract induction as found with Cox regression analysis were BED (with an α/β value of 1.75 Gy and a μ value of 0.75 h^{-1}), allogeneic BMT, and steroid administration for >14 weeks. The relative risk of developing a cataract for patients who received allogeneic BMT and those who received steroid treatment after BMT for >14 weeks was 1.6 and 3.8, respectively. The level of significance

was not reached for age (p = 0.15) and heparin administration for veno-occlusive disease (p = 0.12).

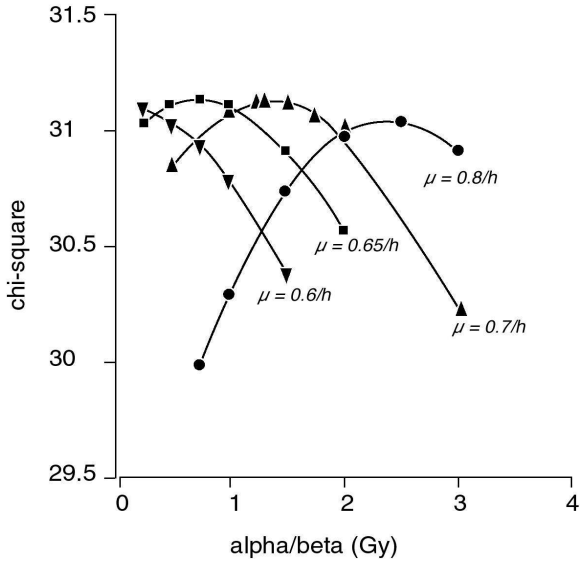

Fig. 1. Chi-square as a parameter of goodness of fit as a function of α/β for different values of μ. STBI data of patients who did not receive steroids or heparin. Maximal chi-square value was obtained for α/β = 0.75 Gy and μ = 0.65 h^{-1}.

When Cox regression analysis was applied for patients who had not received steroid treatment after BMT (n = 324, 115 cataracts), a maximal chi-square value was obtained for α/β = 1 Gy and μ = 0.6 h^{-1}. With covariate analysis, type of graft was not found to be a risk factor (p = 0.08). Therefore, the relative risk for allogeneic BMT of 1.6 found for the data set comprising all 494 STBI patients must have been due to the steroid treatment, which is most frequently given for graft vs. host disease (GVHD) after allogeneic BMT. Heparin administration was now found to be a risk factor (p = 0.03). Cox regression analysis was repeated on the data of patients who had no post-BMT steroid treatment and also no heparin administration (n = 302, 100 cataracts). We found α/β = 0.75 Gy, and μ = 0.65 h^{-1} (Fig. 1). With other risk factors known to interfere excluded, the only risk factor was the BED. Using α/β = 0.75 Gy and μ = 0.65 h^{-1}, we eventually performed Kaplan-Meier analysis to obtain dose-effect relationships.

Dose -effect relationships

Cataract-free survival and cataract incidence were calculated with Kaplan-Meier in different BED subgroups as a function of follow-up time for the 302 patients who were not treated with steroids or heparin after BMT. For α/β = 0.75 Gy and μ = 0.65 h^{-1}, the BED values are in the range of 37.3 Gy (TBI dose = 8.0 Gy, dose rate = 1.14 Gy/h) to 126 Gy (TBI dose = 10 Gy, dose rate = 15 Gy/h).

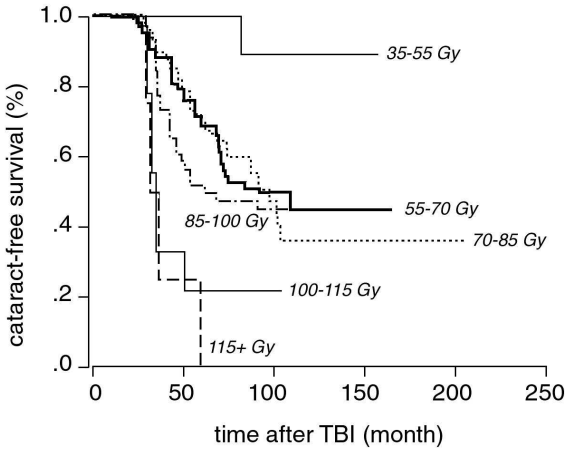

Fig. 2. Cataract-free survival as a function of follow-up time after TBI for BED subgroups. STBI data of patients who did not receive steroids or heparin. BED was calculated for α/β = 0.75 Gy and μ = 0.65 h^{-1}.

The BED subgroups for which the cataract- free survival was calculated, were 35-55 Gy, 55-70, 70-85 Gy, and so forth (Fig. 2). The cataract-free survival in these subgroups did not decrease for interval times >120 month after BMT. For each subgroup, the cataract incidence was therefore calculated at 120 months after BMT. Figure 3 shows the cataract incidence as a function of the BED. The curve could be fitted with a linear function (R = 0.89). The cataract incidence y is a function of x with x = BED using α/β = 0.75 Gy and μ = 0.65 h^{-1}.

Influence of steroid treatment

The cataract incidences per BED interval as shown in Fig. 3 for patients not treated with steroids and heparin are shown again in Fig. 4, curve 1. In addition, a dose-effect the relationship was obtained for patients treated with steroids, irrespective of the duration of steroid treatment (n = 122, 50 cataracts), and no heparin treatment (Fig. 4, curve 2).

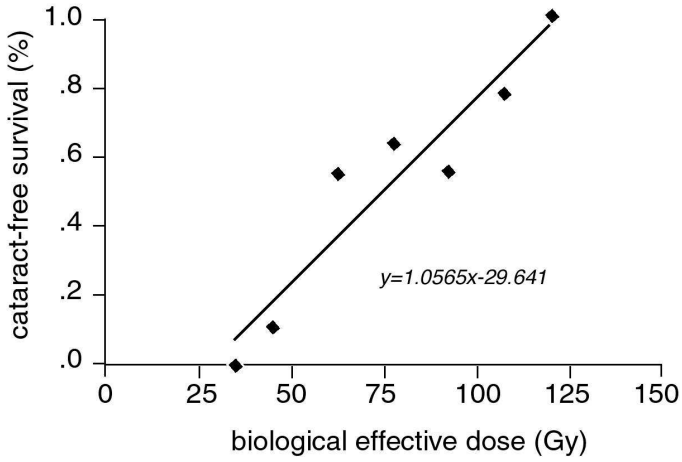

Fig. 3. Cataract incidence of patients who did not receive steroids and heparin as a function of BED for α/β = 0.75 Gy and μ = 0.65 h^{-1} at 120 months after TBI treatment. The solid line is a fit through the data points with R = 0.89. y = cataract incidence; x = BED value.

A dose-effect relationship was also obtained for patients treated with steroids for >14 weeks after BMT and no heparin treatment (n = 29, 20 cataracts; Fig. 4, curve 3). It is clear that for more intensive treatment with steroids, the curve shifts to lower BED values. In addition, analyzing the effect of steroid treatment for >14 weeks on cataract-free period, the median cataract-free period proved to be 40.7 ± 7.4 months; for patients not treated with steroids and heparin it is 102.2 ± 10.9 months (Fig. 5, curves B and A, respectively).

Influence of heparin treatment

A dose-effect relationship was also obtained for patients treated with heparin only and no steroid treatment (n = 21, 14 cataracts). Data points are shown in Fig. 4, curve 4. It is clear that heparin increased the cataract formation compared with patients not treated with heparin and steroids.

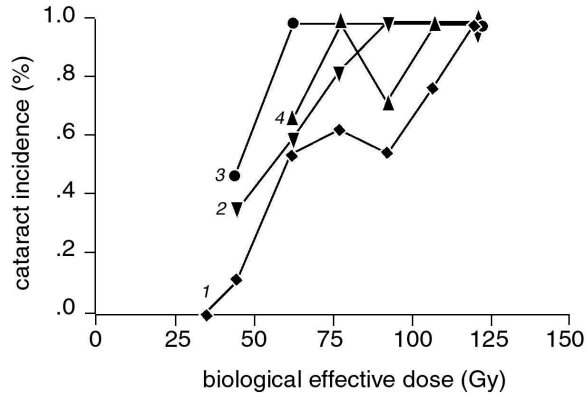

Fig. 4. Cataract incidence as a function of BED (BED for α/β = 0.75 Gy and μ = 0.65 h^{-1} at 120 months after TBI). Curve 1, cataract incidence for patients who did not receive steroids or heparin (n = 302, 100 cataracts); curve 2, patients treated with steroids, irrespective of duration (n = 122, 50 cataracts); curve 3, patients treated with steroids for >14 weeks (n = 29, 20 cataracts); curve 4, patients treated with heparin (n = 21, 14 cataracts).

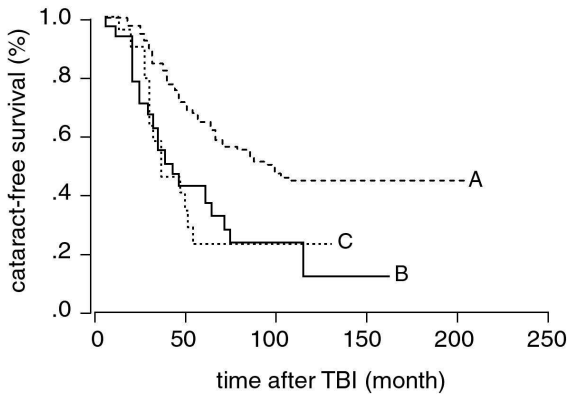

Fig. 5. Cumulative cataract-free survival as function of time after TBI for patients who did not receive heparin or steroids (n = 302, 100 cataracts) (A); patients treated with steroids for >14 weeks and no heparin (n = 29, 20 cataracts) (B); and patients treated with heparin and no steroids (n = 22, 14 cataracts) (C).

The median cataract-free period of patients with only heparin was shorter compared with that of the control group of patients without heparin and steroid treatment, 45.0 ± 10.6 months (Fig. 5C) *vs.* 102.2 ± 10.9 months (Fig. 5A), respectively.

Eye shielding

Cataract incidences at 120 months were calculated for a few TBI schemes using α/β = 0.75 Gy and μ = 0.65 h^{-1}. The cataract incidences for a lens dose reduction of 20% were calculated with the equation shown in Fig. 3. A 20% reduction of the lens dose resulted in a lowering of the BED values of 35% and about a 50% decreased cataract incidence (Table 2).

Table 2. Effect of eye shielding

TBI dose (Gy)	Time (h)	Dose rate (Gy/h)	BED* (Gy)	Cataract incidence **(%)
10	0.93	10.8	120.2	97.3
8***	0.93	8.6	78.5	53.2
7.5	0.5	15.6	75.3	49.9
6***	0.5	12.5	49.2	22.3

*BED calculated with α/β = 0.75 Gy and μ = 0.65 h^{-1}.
** Calculated with the equation Incidence = 1.06xBED – 29.6 (Fig. 3).
*** Eye dose after shielding.
Abbreviations: TBI = total body irradiation; BED = biologic effective dose.

DISCUSSION

The role of TBI in cataract induction after BMT is well documented (1,4,5,7,8,10,16,17). Factors reported to influence cataractogenesis after TBI and BMT are total dose, fractionation scheme, dose rate and steroid therapy (1-9). The influence of these factors on cataract incidence is usually discussed in qualitative terms. A correlation with the BED has not been made previously.

The relationship between cataract formation and exposure to ionizing radiation has been known since 1897 (18). In animal models, radiation-induced cataracts have been reported after exposure to fractionated irradiation (15) and after different types of ionizing radiation (19, 20). In humans, Merriam and Focht (21,22) were the first to describe the relationship between total dose, dose rate and cataract formation. However, only a range was reported for total treatment time and total dose in which the probability of cataract induction varied

between 0 and 1 (23). A relevant way to evaluate the influence of total dose and dose rate of a given TBI treatment on the human eye lens is not presently available.

Linear-quadratic model

A difficulty in determining the dose-effect relationship in cataract formation, taking into account the different radiotherapy, RT, parameters such as total dose, dose per fraction, number of fractions, and dose rate, is the lack of a general theory of how to include these parameters. The LQ-BED concept, which takes all these parameters into account, has already been fruitfully applied in RT for equating different fractionation schemes and for irradiation with different dose rates (11,12). It has proved useful in different clinical situations, including the comparison of fractionation schemes for pain relief in the RT of bone metastases (24, 25), and also for the elucidation of the late effects after irradiation for cervical and rectal carcinoma with different dose rates (26,27). From experimental and clinical data, it is known that the value of α/β is about 10 Gy for early responding tissues and tumors and 1- 4 Gy for late responding tissues (13). The value of μ is about 1 h^{-1} for early responding tissues and ranges between 0.3 and 0.9 h^{-1} for late responding tissues (13).

To develop a possible means to predict the cataractogenic effect of a given TBI schedule, we applied the LQ-BED concept to the EMBT registry data of patients with acute leukemia conditioned with STBI and who underwent BMT in their first or second complete remission. Unknown variables were the tissue-specific parameter α/β and the rate of sublethal damage repair μ. These parameters could be fitted using the large EMBT database, which provided sufficient data for a cataract induction study. In this analysis, we did not use the fractionated TBI data. This proved to be too complex, because a large number of schemes consisted of two or three fractions daily, and the intervals were not known. In addition, another unknown parameter would have to be included to correct for differences in overall treatment time. Using the single-dose treatments, however, we could analyze a reliable homogenous group of patients.

The α/β value of 0.75 Gy for the human eye lens is in the lower range of those found for other late responding tissues (Table 3). Moreover, this value fits well with the α/β value of 1.2 Gy, with 95% confidence interval of 0.6 – 2.1 Gy, derived for cataract induction from a murine lens model (14). The low α/β value indicates that cataract formation can be considered a late effect.

During protracted irradiation, repair of sublethal damage takes place. Therefore, the effectiveness of a dose administered at a low-dose rate is less effective than the same dose delivered with a high-dose rate exposure. The rate of repair for late responding tissues is in the range of 0.2 - 0.9 h^{-1} (median 0.5) (Table 3). The rate of repair derived for cataract development in our study was 0.65 h^{-1}. This value is within the range of the values reported for other late responding tissues (Table 2).
Using the LQ-BED concept, previous statements about influence of dose rate and total dose on cataract induction now fall into place. In general, TBI delivered in a single fraction and using a high dose rate results in a high BED, and hence a higher probability of cataract formation. In contrast, the use of low dose rate results in a low BED and in a lower cataract incidence, even when the TBI dose is higher than a TBI dose administered at a high dose rate. Some examples are shown in Table 3. Because α/β and μ are tissue-specific and independent of the irradiation regimen, the values of α/β and μ derived from STBI data are also applicable for fractionated radiotherapy.

Influence of transplant type

In the present study, the cataract incidence was significantly higher after allogeneic than after autologous BMT (relative risk = 1.6). However, the influence of allogeneic BMT on cataract incidence was apparently not due to the type of graft itself. After excluding patients treated with steroids, the type of graft was no longer a risk factor. The additive effect of steroid administration in allografted patients was also clearly demonstrated in the Seattle (1,17), Basel (5) and Utrecht (10) experiences. Hence, the impact of allogeneic BMT is strongly interrelated with the effects of steroid administration for treatment of acute or chronic GVHD. In our population, 80% of the patients who were treated with steroids after BMT for >14 weeks had developed acute and or chronic GVHD.

Influence of steroid administration

The cataractogenic effect of steroids has been reported in patients with renal transplantation and after steroid treatment for rheumatoid arthritis (28,29). In patients receiving high-dose therapy without TBI before BMT, steroid therapy has also been

reported to be cataractogenic (1). In patients who had TBI as a part of their conditioning regimen, steroids were reported to have an additive or a synergistic effect on cataractogenesis (1,5,7,8,10,17,30). Tichelli et al. (5) observed in univariate analysis that steroid administration for >100 days and the development of chronic GVHD significantly increased cataract incidence. However, when adjustments were done in multivariate analysis, they found only steroid administration of >100 days was an independent factor; the impact of chronic GVHD disappeared. In the present study, the covariate analysis revealed steroid therapy as an independent factor influencing cataract induction. The dose-response curve in Fig. 4 (curve 3) illustrates the deleterious effect of long-lasting steroid treatment. In addition, the median cataract-free period was significantly shorter (Fig. 5B) compared with that of the control group not treated with steroids and heparin (Fig. 5A).

Influence of heparin administration

In a single-institution retrospective analysis of 494 TBI patients, heparin was administered for prevention of veno-occlusive disease at a dose of 1 mg/kg during a mean period of 1 month. Heparin was found as a lens protector against radiation in patients treated with either single or fractionated TBI schemes (8). Also from the analysis of the EBMT database, Belkacémi et al. (7) concluded that the administration of heparin appears to protect the lens against radiation in the whole population and in the STBI group. However, in the present study, the analysis of the same STBI data of the EBMT database, using the BED concept rather than the total dose, could not confirm the protective effect of heparin (Fig. 4). This finding warranted a new analysis which found a protective effect for heparin when considering STBI and fractionated TBI data of the EBMT database in its entirety (1063 patients), with an incidence of cataract formation of 16% in patients receiving heparin (31 of 195) *vs.* 26.5% (224 of 845) in those who did not receive heparin. Also, from the multivariate analysis of the STBI group, it was previously concluded that heparin protects the lens (7). Because this result was not confirmed by the analysis using BED values, a reanalysis of the original data (7) was performed and revealed a statistical error. After correction, heparin was significantly associated with a higher probability of cataract in the STBI data set. With the present analysis, using BED, it is now clear that the low percentage of cataract formation, 16%, in patients receiving heparin in the STBI and fractionated TBI group (n = 195) correlated with a low mean BED value of 43.2 Gy. The high percentage of 60% of cataract induction among the heparin-treated patients in the STBI group (18 cataracts in 30 patients who received heparin), however, correlated with a

high mean BED value of 85.1 Gy. In addition, the median cataract-free period of the heparin-treated patients was shorter than that of the control group of patients without heparin and steroid treatment (45.0 ± 10.6 months [Fig. 5C] vs. 102.2 ± 10.9 months [Fig. 5A], respectively). Heparin in BMT is primarily used for prevention of VOD. Like steroids, heparin probably has access to the lens through diffusion from the surrounding fluid in the eye. How heparin could influence cataract formation is unknown.

Influence of age

The influence of age on cataract induction in patients conditioned with TBI before BMT is controversial. Most investigators did not find any influence of age (1, 7, 5, 31). Fife *et al.* (32), however, reported age as a risk factor for severe cataract requiring surgery after STBI. In the previous EBMT database analysis, age >23 years was not a significant factor in univariate analysis. However, it became significant once adjustments were made for other factors in the multivariate analysis (7).
In the present study with multivariate analysis, which included the BED as risk factor, age >23 years was not associated with a higher risk of cataract induction.

Eye shielding

In most centers, eye shielding during TBI is not performed, because the orbit and central nervous system are regarded as potential sites of residual disease. However, a large variety of TBI schemes with varying, sometimes relatively low, BED values have been applied before BMT. No particular TBI schedule has been reported, to our knowledge, to be associated with an increased risk of central nervous system relapse after completed treatment of leukemia. Therefore, the risk and benefit of eye shielding during TBI to reduce the incidence of cataract formation should be investigated. Our results suggest that a reduction of the lens dose by 20% is associated with a 50% reduction in cataract incidence. This is due to the lower TBI dose and also results from the dose rate reduction (double advantage), resulting in BED values that are reduced by about 35%. Nevertheless, the BED values obtained after this dose reduction are still in the range of those calculated for several TBI schemes used by members of the EBMT. Therefore, in those cases in which the treatment time is relatively short (high-dose rate irradiation), the BED is high, and there is no overt risk of central nervous system recurrence, eye shielding may be considered. A study to investigate the effect of eye shielding in more detail is in progress.

Chapter 5

CONCLUSIONS

This retrospective study performed in a large number of patients, who received STBI as part of the conditioning before BMT, has resulted in a dose-effect relationship for cataract formation. The use of BED instead of the total dose enabled the incidence of cataract formation to be predicted in a reasonably consistent way.

Cox regression analysis applied to patients who did not receive steroid treatment revealed a maximal chi-square value for $\alpha/\beta = 1$ Gy and $\mu = 0.6\ h^{-1}$. For a homogeneous population who received neither steroids nor heparin, the calculated α/β value was 0.75 Gy. This value is in the lower range of those found for other late responding tissues. The rate of sublethal damage repair μ for cataract development was found to be $0.65\ h^{-1}$.

The analysis confirmed the influence of steroid administration on cataract formation. Heparin administration in STBI patients increased cataract formation, and age was not found to be a risk factor.

A measure to diminish the probability of cataract formation, apart from minimizing steroid treatment, is the application of a TBI scheme with a BED appropriate for eradication the disease, as well as for minimizing late effects. Eye shielding needs to be studied as medium to lower the BED for the eye lens.

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Chapter 5

Chapter 6

RENAL DYSFUNCTION AFTER TOTAL BODY IRRADIATION:

DOSE-EFFECT RELATIONSHIP

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Chapter 6

ABSTRACT

Purpose: Late complications related to total body irradiation (TBI) as part of the conditioning regimen for hematopoietic stem cell transplantation have been increasingly noted. We reviewed and compared the results of treatments with various TBI regimens and tried to derive a dose-effect relationship for the endpoint of late renal dysfunction. The aim was to find the tolerance dose for the kidney when TBI is performed.

Methods and Materials: A literature search was performed using PubMed for articles reporting late renal dysfunction. For intercomparison, the various TBI regimens were normalized using the linear-quadratic model, and biologically effective doses (BEDs) were calculated.

Results: Eleven reports were found describing the frequency of renal dysfunction after TBI. The frequency of renal dysfunction as a function of the BED was obtained. For BED > 16 Gy an increase in the frequency of dysfunction was observed.

Conclusions: The tolerance BED for kidney tissue undergoing TBI is about 16 Gy. This BED can be realized with highly fractionated TBI (e.g., 6 x 1.7 Gy or 9 x 1.2 Gy at dose rates > 5 cGy/min). To prevent late renal dysfunction, the TBI regimens with BED values > 16 Gy (almost all found in published reports) should be applied with appropriate shielding of the kidneys.

INTRODUCTION

Total body irradiation (TBI) is a well-established approach for conditioning patients with leukemia for hematopoietic stem cell transplantation (HSCT). Improvements in patient care, conditioning regimens, TBI techniques, and posttransplant care have led to better patient outcomes and longer patient survival. When survival improved, late complications from the treatment became important for patients' quality of life after transplantation. Treatment-related morbidity results from injury caused by graft versus host disease, infection, cytotoxic agents, and ionizing radiation. Eye lenses, lungs, liver, and kidneys are particularly susceptible to injury from radiation. In addition to the acute side effects as a consequence of TBI, severe late effects such as pulmonary dysfunction and renal toxicity must be considered. Several authors have concluded that TBI probably was the principal cause of late deterioration of renal function after HSCT (1-5). Late renal dysfunction is mainly attributable to radiation nephropathy, which is characterized by an increase of serum creatinine, proteinuria, anemia, and hypertension (6,7). The clinical manifestations generally occur 1- 1.5 years after TBI.

The tolerance dose associated with a 5% risk of renal dysfunction at 5 years after single, whole kidney irradiation is about 23 Gy (8) and 20 Gy if both kidneys are irradiated (9). The tolerance dose after fractionated TBI is probably lower (9-13), but the optimal dose has not yet been established. TBI for HSCT is delivered with different treatment regimens at the different centers: fractionated or hyperfractionated radiotherapy or as a single fraction, all with varying dose rates. For intercomparison of the TBI schedules, we applied the linear-quadratic (LQ) concept that allows converting each TBI schedule into a single biologically effective dose (BED) value for kidney tissue (14).

The purpose of this study was to evaluate the incidence of late renal dysfunction secondary to TBI as a function of the BED. The aim was to find the tolerance dose for the kidney when TBI is performed as part of the conditioning regimen for HSCT.

METHODS AND MATERIALS

Literature Search

A literature search was conducted in PubMed to identify English-language studies, using the search terms TBI, bone marrow transplantation, hematopoietic stem cell, kidney/renal failure/insufficiency/dysfunction, and late effects. Cross-referencing of the relevant articles identified additional reports. The studies were included if the follow-up time was at least 1

year. Studies were excluded when the frequency of renal dysfunction could not be related to a specific TBI regimen or when the number of treated patients was < 10.

LQ and BED concept

The occurrence of a biologic effect, E, depends on the dose in a linear and quadratic fashion: $E = n (\alpha d + \beta d^2)$, where n is the number of fractions, d is the dose per fraction, and α and β are the parameters that determine the initial slope and curvature of the underlying cell-survival curve (14,15). From that equation, the BED can be derived (14): $BED = nd [1+d/(\alpha/\beta)]$.

For low-dose-rate irradiation, $BED = nRT [1 + kR/(\alpha/\beta)]$, where R is the dose rate, T is the treatment time per fraction, and $k = 2[1-\{1-\exp(-\mu T)\}/(\mu T)]/\mu$. The factor k depends on the sublethal damage repair rate μ during low-dose-rate irradiation and treatment time T. The parameter μ is related to the half-time for monoexponential repair of sublethal damage T½, where $T½ = \ln 2/\mu$ (16,17).

The strength of the LQ-BED concept is that for high- and low-dose-rate treatments, the BED values resulting in a specific isoeffect, in our case a certain percentage of renal dysfunction, are equal.

The value of α/β is about 10 Gy for acute-reacting tissues, and 1 to 4 Gy for late-reacting tissues. The rate of sublethal damage repair, μ, is about 1 h^{-1} (T½ = 0.7 h) for acute-reacting tissues such as the skin and mucosa and ranges between 0.3 to 0.9 h^{-1} (T½ = 2.3 - 0.8 h) for late-reacting tissues, such as the lungs and central nervous system (15-17). The human kidney is also considered to be a late responding tissue. For late kidney damage, α/β values of 2 - 3 Gy have been reported from the responses of experimental animals (18,19), a value similar to that observed in other late-responding tissues (20).

Conversion of TBI schemes to BED values

We extracted the total doses, fraction doses, number of fractions, and dose rates reported in the studies. If the treatment time per session was reported, the mean dose rate was used. When a range of dose rates was given, the mean or median dose rate was used. For the endpoint of late renal dysfunction, we applied an α/β of 2.5 Gy and μ of 0.35 h^{-1} (corresponding to a half-time of 2 h for monoexponential repair). We assumed that for those TBI regimens for which the interfraction interval was not reported, the interfraction interval was 4 h.

Endpoint and dose-effect relationship

Late renal dysfunction was the analyzed treatment endpoint. Late renal dysfunction is mainly attributable to radiation nephropathy, which is characterized by an increase of serum creatinine, proteinuria, anemia, and hypertension. The definitions of renal dysfunction as reported are tabulated in Table 1.

For the endpoint of late renal dysfunction, a dose-effect relationship as a function of the BED was obtained. Linear regression analysis was applied using the Statistical Package for Social Sciences, version 10.1 (SPSS, Chicago, IL) statistical packet.

Table 1. Renal dysfunction definitions

Authors	Definition of late renal dysfunction
Frisk et al. (1)	**GFR < 70 mL/min/1.73 m^2 > 6 mo after HSCT**
Lawton et al. (2)	**Increased (BUN) and creatinine, anemia, hypertension**
Lönnerholm et al. (4)	**>25% decrease in GFR**
Van Why et al (5)	**Doubling of baseline creatinine, or clearance of 50mL/min/1.73 m^2**
Rabinowe et al (7)	**Hemolytic-uremic syndrome**
Miralbell et al. (11)	**Increase of serum creatinine level to > 110 µmol/L**
Chou et al. (12	**Mildly elevated BUN and creatinine; failure requiring dialysis**
Borg et al. (21)	**Serum creatine level > 120 µmol/L**
Bradley et al. (22)	**Requiring dialysis, or having elevated renal function tests**
Tarbell et al. (23)	**Elevated BUN and creatinine**
Igaki et al. (24)	**Serum creatine level > 1.1 mg/dL**

Abbreviations: GFR = glomerular filtration rate; HSCT = hematopoietic stem cell transplantation; BUN blood urea nitrogen.

RESULTS

Eleven reports were found describing the frequency of late renal dysfunction after TBI (1, 2,4,5,7,11,12,21-24). Randomized trials comparing the results of two or three TBI regimens with respect to renal damage were not found. In only three reports was the frequency of renal dysfunction and nephropathy as a function of the TBI dose noted (2,11, 24). Several studies (3,25-27) could not be included because of obscurity of the dose rate, a low number of patients, or the relationship between the dose and renal dysfunction was not indicated.

We collected the TBI schemes and calculated the matching BED values for kidney tissue (Table 2). The dose rate for the TBI treatment reported by Van Why *et al.* (5) was estimated by us to be 14 cGy/min, within the dose rate range of other fractionated treatments, because of the small fraction size and short treatment time in which the repair of sublethal damage could only have been marginal. The exact magnitude of the dose rate, however, for such a small fraction dose is of less importance. The chemotherapeutic drugs used for conditioning are listed in Table 3.

Table 2. Summary of studies with TBI schemes, frequency of renal dysfunction, and biologically effective dose of kidney

Authors	Patients (n)	Fractions (n)	Fraction dose to kidney (Gy)	Dose rate (cGy/min)	Dysfunction (%)	BED_{kidney} (Gy)
Frisk et al. (1)	**26**	**1**	**7.5**	**15**	**26.9**	**28.0**
Lawton et al. (2)	**72**	**9**	**1.55**	**14**	**29**	**22.4**
Lawton et al. (2)	**68**	**9**	**1.32**	**11.9**	**14**	**18.0**
Lawton et al. (2)	**17**	**9**	**1.09**	**9.8**	**0**	**14.0**
Lönnerholm et al. (4)	**41**	**1**	**7.5**	**15**	**24.4**	**27.9**
VanWhy et al. (5)	**39**	**8**	**1.65**	**14***	**43**	**21.7**
Rabinowe et al. (7)	**112**	**6**	**2**	**7.5**	**9.8**	**21.1**
Miralbell et al. (11)	**24**	**6**	**1.67**	**16**	**5**	**16.6**
Miralbell et al. 11)	**32**	**6**	**2**	**16**	**26**	**21.4**
Miralbell et al. (11)	**23**	**6**	**2.25**	**16**	**45**	**25.3**
Chou et al. (12)	**58**	**6**	**2**	**15**	**10**	**21.4**
Chou et al. (12)	**15**	**1**	**7**	**15**	**25**	**24.9**
Borg et al. (21)	**59**	**6**	**2**	**7.5**	**15**	**21.1**
Bradley et al. (22)	**77**	**9**	**1.5**	**12**	**9**	**21.4**
Tarbell et al. (23)	**12**	**8**	**1.75**	**10**	**33.3**	**23.5**
Tarbell et al. (23)	**15**	**6**	**2**	**10**	**46.7**	**21.2**
Igaki et al. (24)	**70**	**6**	**2**	**10****	**21.5**	**21.2**
Igaki et al. (24)	**39**	**6**	**1.7**	**8.5**	**0**	**16.5**

Abbreviations: TBI = total body irradiation; BED_{kidney} = biologically effective dose of kidney.
*Dose rate estimated by us.
**Personal communication (Dr. Igaki, e-mail December 2005).

Table 3. Chemotherapeutic drugs used for conditioning

Authors	Chemotherapeutic drugs
Frisk et al. (1)	Teniposide, daunorubicin, vincristine, cyclo and cytarabin
Lawton et al. (2)	Cytarabine, cyclophosphamide
Lönnerholm et al. (4)	ALL: teniposide, vincristine, daunorubicin, cytarabine, cyclophosphamide AML: cyclophosphamide, cyclophosphamide and busulfan; HD: BCNU, etoposide, cytarabine, cyclophosphamide; or BCNU, etoposide, methyl-GAG, cyclophosphamide; NHL: BCNU, etoposide, cytarabine, cyclophosphamide; MM: cyclophosphamide, melphalan
VanWhy et al. (5)	Cyclosporin, amphotericin B
Rabinowe et al. (7)	Cyclophosphamide; cyclophosphamide and cytosine arabinose
Miralbell et al. (11)	Cyclophosphamide; cyclophosphamide and thiotepa, or daunorobicin, or busulfan, or cytarabine
Chou et al. (12)	Combinations of cyclophosphamide, Ara-C, methotrexate, etoposide
Borg et al. (21)	Cyclophosphamide; cyclophosphamide and melphalan
Bradley et al. (22)	Cyclophosphamide; cyclophosphamide and teniposide; etoposide
Igaki et al. (24)	Cytosine arabinoside and cyclophosphamide; busulfan and cyclophosphamide
Tarbell et al. (23)	ALL: cyclophosphamide, Ara-C, teniposide; neuroblastoma: teniposide, cyclophosphamide, cis-platinum, melphalan; methotrexate

Abbreviations: Ara-C = arabinoside; BCNU = carmustine; Methyl-GAG = methylglyoxal-bis(guanylhydrazone); ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukaemia; MM = multiple myeloma; NHL = non-Hodgkin's lymphoma; HD = Hodgkin's disease.

The frequency of renal dysfunction as function of the BED derived from the 11 reports is shown in Fig. 1. Linear regression analysis was performed. The intercept of the line through the data points occurred at a BED of 18.2 Gy (standard error 1.3 Gy). However, for deterministic effects, such as renal dysfunction, an S-shape curve is expected. From Fig.1, one can read that the frequency of late renal damage is practically zero for BED

values < 16 Gy. Therefore, using the clinical data for late renal dysfunction at the lowest BED values, we estimated a threshold dose of about 16 Gy. For BED_{kidney} values > 16 Gy, the frequency of late renal damage increases to about 10- 47% at a BED of 21-22 Gy (Table 2). TBI schemes with a BED_{kidney} of <16 Gy were the fractionated scheme of nine fractions of 1.55 Gy TBI with renal shielding to 1.2 Gy/fraction, as can be derived from the data of Lawton *et al.* (2) (Table 2), or six fractions of 2 Gy with renal shielding to 1.7 Gy/fraction (24), provided that the fraction dose was administered within 30 min (i.e., at dose rates of > 5 cGy/min).

DISCUSSION

Total body irradiation is an important part of the conditioning regimen for HSCT. Several authors have concluded that TBI is the principal cause of late deterioration of renal function after HSCT (1-5). TBI is delivered with many different treatment regimens, including fractionated or hyperfractionated radiotherapy or as a single fraction, all with varying dose rates. Nevertheless, in the published reports, the results of HSCT after conditioning with chemotherapy and TBI have been compared without considering the differences in regimens with respect to TBI. Several authors have tried to find a relationship between the total doses of the TBI and treatment outcome. Vriesendorp *et al.* (28) stated that the important issues in any analysis of TBI results are the dose, fraction size and endpoint selection and that different TBI procedures using a different total dose and fraction size could not be compared without radiobiologic “normalization” because every dose/fractionation scheme has a different effect on the various tissues. This normalization would also be different for different endpoints (e.g., for acute and late effects, different α/β values are applied). We used the LQ concept, which allows converting each TBI schedule into a single BED (14), for intercomparison of the TBI schedules with respect to late renal dysfunction. For the endpoints of cataract induction of the eye lens after TBI and prevention of keloid after surgical resection, we have earlier reported the dose-effect relationships using the LQ-BED concept (29,30).

In only three reports was the frequency of renal dysfunction as a function of the TBI dose mentioned (2,11,24). It was shown that for renal doses > 10 Gy, the incidence of late renal damage increased to 30-45% at TBI doses of about 14 Gy, applied in six or nine fractions. Lawton *et al.* (2) and Juckett *et al.* (31) of the same institute reported that treatment with

renal shielding to a dose of 9.8 Gy (six fractions of 1.63 Gy) reduced the decline in renal function compared with treatment without shielding. In addition, Igaki *et al.* (24) also showed that renal shielding to 10 Gy in six fractions reduced the incidence of late renal failure to 0% compared with six fractions of 2 Gy with 21.5% rate of late renal dysfunction. The TBI dose of 10 Gy in six fractions at a dose rate of 8.5 cGy/min (24) corresponds to a BED for the kidney of 16.5 Gy. From the dose-effect relationship (Fig. 1), we have estimated that the tolerance BED for the kidney is about 16 Gy. A BED of about 16 Gy can be realized with fractionated TBI of six fractions of 1.7 Gy or nine fractions of 1.2 Gy at dose rate > 5 cGy/min. Because the treatment time at these dose rates is < 30 min, repair of sublethal damage does not play an important role in the BED. This tolerance dose of about 10 Gy is much lower than that found after single, whole kidney irradiation with external-beam therapy applied without the use of any additional therapy, such as in TBI. The tolerance dose associated with a 5% risk of renal dysfunction at 5 years after fractionated single whole kidney irradiation is about 23 Gy, corresponding to a BED of 41.4 Gy (8) and 20 Gy (BED 36 Gy) if both kidneys are irradiated (9). An explanation for the difference in these tolerance doses could be the contribution of the intensive chemotherapy regimens used before and as part of the conditioning for HSCT, as well as that the patient is immunocompromised, temporarily in a poor condition, and susceptible to graft vs. host reactions. This may potentiate the irradiation, as has been shown for some chemotherapeutic agent in experimental HSCT nephropathy (32,33). In addition, Frisk *et al.* (1) observed that the 7 of their 26 patients who developed renal impairment had received more nephrotoxic antibiotics during the early posttransplant period. Administration of potentially nephrotoxic contrast agents used in radiotherapy planning for the TBI may also be responsible for renal dysfunction (34). It must be noted that after intensive chemotherapy only, abnormalities of renal function have also occurred (35,36).

The high incidence of renal dysfunction of 46.7% at a BED of 21.2 Gy (Fig. 1), reported by Tarbell *et al.* (23) may have been because patients with neuroblastoma were involved who had undergone previous therapy with multiple chemotherapeutic agents. The high incidence of 43% at a BED of 21.7 Gy (5) may have resulted from the use of cyclosporin and amphotericin B, drugs not used in other protocols (Table 3). In contrast, the relatively low incidences at a BED of 28 Gy may have beeen because patients received autologous bone marrow (1,4). In addition, Rabinowe *et al.* (7) reported a relatively low frequency of late renal toxicity (9.8% at a BED of 21.1 Gy) in their patients treated with autologous bone marrow. Glynne *et al.* (25) concluded that renal dysfunction is an uncommon long-term

complication of autologous bone marrow transplantation after single TBI doses of 9.5-11.5 Gy at a dose rate of 4 cGy/min (BED range, 33.4-44 Gy). In almost all other reported studies, most patients received allogeneic bone marrow and no distinction was made between the results for late nephrotoxicity concerning patients with acute lymphoblastic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, or other hematologic diseases. In these patients, graft vs. host disease and, as a consequence, a temporarily poor condition may play a role in the occurrence of renal dysfunction.

With respect to shielding, it must be noted that this will not only result in a lower BED for the shielded organ, but will also result in a lower BED for the leukemic cells and bone marrow present in the tissues in the shadow of the shielding blocks. Thus, disease recurrence may develop in the shielded regions. In this respect, the report of Igaki *et al.* (24) is of interest. Igaki *et al.* (24) used selective renal shielding blocks. The kidneys received a total dose of 10 Gy in six fractions of 1.7 Gy at a dose rate of 8.5 cGy/min (Table 2). Evidence was provided that renal dysfunction was reduced to 0% at this dose level of 10 Gy compared with a 21.5% rate of renal dysfunction with a TBI of six fractions of 2 Gy and a dose rate of 10 cGy/min, without shielding. In addition, overall survival in the shielded patients was not decreased compared with that of the unshielded ones. It must, however, be noted that this was the only one report in which shielding and its effect on survival was discussed - more data are needed.

In conclusion, the tolerance BED for kidney is about 16 Gy. The frequently applied TBI of six fractions of 2 Gy at dose rates of 5-15 cGy/min without renal shielding leads to a BED of about 21-22 Gy. Renal impairment can be expected in >10% of the cases; therefore, TBI of six times 2 Gy should not be applied without shielding. TBI regimens with a BED for kidney tissue of ≤16 Gy, which can be applied without shielding, include six fractions of 1.7 Gy or nine fractions of 1.2 Gy at a dose rate of > 5 cGy/min. All other regimens with BED values > 16 Gy (i.e. almost all schemes found in published studies) should be used with appropriate shielding of the kidneys.

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Chapter 7

BIOLOGICALLY EFFECTIVE DOSE IN TOTAL BODY IRRIDIATION AND HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Chapter 7

ABSTRACT

Background and Purpose: Total body irradiation (TBI) is an important part of the conditioning regimen for hematopoietic stem cell transplantation (HSCT) in patients with hematological malignancies. The results after treatment with various TBI regimens were compared, and dose-effect relationships for the endpoints relapse incidence, disease-free survival, treatment-related mortality, and overall survival were derived. The aim was to define requirements for an optimal treatment schedule with respect to leukemic cell kill and late normal-tissue morbidity.

Materials and Methods: A literature search was performed. Three randomized studies, four studies comparing results of two or three TBI regimens, and nine reports with results of one specific TBI regimen were identified. Biologically effective doses (BEDs) were calculated. The results of the randomized studies and the studies comparing results of two or three TBI regimens were pooled, and the pooled relative risk (RR) was calculated for the treatments with high BED values versus treatments with a low BED. BED-effect relationships were obtained.

Results: RRs for the high BED treatments were significantly lower for relapse incidence, not significantly different for disease-free survival and treatment-related mortality, and significantly higher for overall survival. BED-effect relationships indicate a decrease in relapse incidence and treatment-related mortality and an increase in disease-free survival and overall survival with higher BED values.

Conclusion: "More dose is better", provided that a TBI setting is used limiting the BEDs of lungs, kidneys, and eye lenses.

INTRODUCTION

Total-body irradiation (TBI) is an important part of the conditioning regimen for hematopoietic stem cell transplantation (HSCT) (4,7,10,11,14,20,22,28,36,41). Several authors tried to find a relationship between the total doses of TBI and treatment outcome. Some reported a higher overall survival with increasing TBI dose (28,36), others found opposite results (4,7). Vriesendorp et al. (45) stated that the important issues in any analysis of TBI results are dose, fraction size and endpoint selection and that different TBI procedures could not be compared without radiobiological "normalization". This normalization will also be different for different endpoints.

We reviewed the results of 16 publications describing 14 different TBI regimens and applied, for normalization, the linear-quadratic (LQ) concept, which allows converting each TBI schedule into a single biologically effective dose (BED) (2).

The aim is to evaluate relationships between BEDs and the endpoints: relapse incidence, disease-free survival, treatment-related mortality, and overall survival. The ultimate aim is to derive a treatment regimen that could be recommended, taking a high efficacy and a low toxicity profile into account.

MATERIAL AND METHODS

Literature Search

We searched PubMed from 1986 up to 2005 using the search terms TBI, acute leukemia (AL), allogeneic, and bone marrow transplantation. Studies were included when the follow-up time was at least 3 years. Results on autologous-transplanted patients were excluded because, in general, results concerning relapse rate and treatment-related mortality differ from those after allogeneic transplantation (e.g., no graft-versus-host disease). In only a few instances, also data of some patients treated for chronic myeloid leukaemia, non-Hodgkin's lymphoma, and multiple myeloma were included. Many factors determine the outcome of HSCT, amongst all, status of the disease at transplantation and conditioning regimen. We assumed that these factors are not very different per center, just as the donor type and the percentage of patients who had graft-versus-host disease after transplantation.

Linear-Quadratic and Biological Effective Dose (LQ-BED) Concept

The occurrence of a biological effect E depends on the dose in a linear and a quadratic fashion:

$$E = n(\alpha d + \beta d^2) \quad (1),$$

where n is the number of fractions, d is the dose per fraction at a high dose rate, α (indicative of intrinsic radiosensitivity) and β (indicative of repair capacity) are constants. From equation (1), the BED can be derived (2):

$$BED = nd[1+d/(\alpha/\beta)] \quad (2).$$

For fractionated low-dose-rate irradiation:

$$BED = nRT[1 + kR/(\alpha/\beta)] \quad (3),$$

where R is the dose rate, T the treatment time of a dose fraction, and
$k = 2(1-\{1-\exp(-\mu T)\}/(\mu T)]/\mu$ (12). The μ is related to the half-time for repair of sublethal damage T½, where $T½ = \ln 2/\mu$.

For the leukaemia-related endpoints we applied α/β = 10 Gy and μ = 1.4/h (40,42). For the endpoints transplant-related mortality and overall survival we applied the same values; transplant-related mortality is related to the chemoradiotherapy used for conditioning: early toxicity, and to the infusion of donor marrow: bone marrow aplasia, graft failure/rejection, acute graft-versus-host disease, and lung toxicity.

For the TBI regimens the overall treatment times ranged from < 1 h (single dose) to 6 days (e.g., seven daily fractions of 2.25 Gy). Due to the relatively short overall treatment time, long cell-cycle time (9,34) and potential doubling time (40), a correction for overall treatment time was not applied.

Dose-Effect Relationships, Relative Risks

Linear regression of the dose-effect relations weighted by the number of patients was applied using SPSS 9.0 Statistical Package. The relative risks (RRs) of a high-BED versus a low-BED treatment were calculated. An RR of 1 indicates no difference in endpoints for a high-BED versus a low-BED treatment. Subsequently, we calculated the pooled RR. RRs, pooled RR, and 95% confidence intervals were calculated using STATA 8.0. When a 95% confidence interval did not include the value 1, we considered the RR significantly different from 1.

Late normal tissue morbidity

As a theoretical exercise we evaluated the effect of the TBI regimens on some late responding tissues, and calculated the corresponding BED values for eye lenses, lungsand kidneys, supposing that no shielding of the organs was applied.

For late responding tissues the BED value can be calculated using the appropriate α/β and μ values. Earlier, we derived an α/β = 0.75 Gy and μ = 0.65/h for cataract development (44). For lung and kidney we applied α/β values of 3.5 Gy and 2.5 Gy, respectively, and for μ the value of 0.46/h (40,42).

RESULTS

Literature Search

Three randomised studies were identified (7,14,20), and four studies in which results of two or three TBI regimens were compared (4,11,28,36) (Table 1). Nine papers were found with results of one specific TBI scheme (1,3,10,14,18,21,30,33,41) (Table 1). Several studies could not be included because of obscurity of dose rate or number of fractions, abundance of autologous-transplanted patients, or because of a too short follow-up period (6,13,15-17,35,35,37,38,43).

Biologically Effective Doses, Relative Risk

Table 1 also shows the calculated BED values for leukemic cell kill and organs at risk, as well as the percentages of the four treatment endpoints.

In Table 2, the total number of patients and responders for the low- and higher-BED treatments are presented. The RRs with 95% confidence limits are shown as well. The RRs of the randomised trials (7,14,20) were not significantly different from 1, except for relapse incidence in one study (7). We therefore decided to pool the results of the three randomized studies with those of studies in which two or three TBI regimens were compared (4,11,28,36). For all but one study, the relapse incidence was lower for the high-BED treatments (RR < 1). For the pooled data, the relapse incidence was significantly lower for the high-BED treatments compared with low-BED treatments.

Disease-free survival was increased for all the regimens with the high-BED treatments, except for one. The pooled data show that for the high-BED treatments the disease-free survival was not significantly higher than for the low-BED treatments.

Treatment-related mortality was higher for the high-BED treatments in three studies (4,7,20) and lower in five studies (4,11,14,28,36). For the pooled data, the treatment-related mortality was not significantly lower for the high-BED regimens than for the low-BED schemes.

Table 1. Summary of publications with number of patients, TBI fractionation scheme, dose rate, calculated BED for leukemic cells, lung, eye lens and kidney, relapse incidence, disease-free survival, treatment-related mortality and overall survival. ALL: acute lymphoblastic leukemia; ANLL: acute nonlymphoblastic leukemia; BED: biologically effective dose; TBI: total-body irradiation.

Reference	Patients (n)	TBI scheme	Dose rate (Gy/h)	BED leukemia (Gy)	Relapse incidence (%)	Disease free survival (%)	Treatment related mortality (%)	Overal survival (%)	BED,lung,lens, kidney (Gy)
Randomized trials:									
(7]	34	6x2 Gy	3.9	13.9	35	58	12	65	18.3;40.5;20.8
(7]	36	7x2.25 Gy	3.9	18.5	12	59	32	59	25.1;57.2;28.7
(14]	27	1x10 Gy	3.0	13.4	22.2		55.6	33	28.2;82.8;35.5
(14]	26	6x2 Gy	3.0	13.8	11.5		30.8	54	18.2;39.8;20.7
(20]	73	1x10 Gy	2.7	13.1	27		33	38	27.4;78.9;34.4
(20]	74	11x1.35 Gy	15	16.8	15		35	45	20.5;41.1;22.8
Studies comparing two or three TBI schemes:									
(4]	24	6x1.67 Gy	9.6	11.6	25	42	21	62	14.7;31.5;16.5
(4]	66	6x2 Gy	9.6	14.2	15	50	29	56	18.7;42.8;21.4
(4]	26	6x2.25 Gy	9.6	16.2	38	38	19	46	21.9;52.0;25.2
(11]	53	3x3.3 Gy	3.6	12.1	31		41	51	18.1;46.1;21.3
(11]	46	6x2 Gy	3.6	13.9	13		31	68	18.3;40.5;20.8
(28]	36	1x7.5 Gy	15.6	12.0	19	56	30.5	58	21.9;71.7;27.6
(28]	48	8x1.65 Gy	6.0	15.1	10	69	23	77	19.1;40.1;21.4
(36]	65	1x10 Gy	2.34	12.8	29	43	38	45	26.3;73.6;32.9
(36]	106	6x2 Gy	3.96	13.9	26	56	24	64	18.3;40.8;20.9
Studies reporting results of one TBI scheme or from which results were combined:									
(1]	57	5x2.2 Gy	3.6	12.9		47		53	17.3;39.3;19.8
(3]	104, ANLL	1x10 Gy and 6x2 Gy	4.2	14.1	31.3	49.5	27.5	51	30.6;95.1;38.8
(3]	84, ALL	1x10 Gy and 6x2 Gy	4.2	14.1	53.2	33.2	26	34.6	18.4;41.1;21.0
(10]	65	6x2 Gy	3.6	13.9		65	23	45	18.3;40.5;20.8
(14]	22	1x9.2 Gy	3.0	12.2	13.6		36.4	54	25.1;73.4;31.5
(18]	166	1x5 Gy	34.8	7.34	44	29		31.9	12.0;37.4;14.8
(21]	39, ANLL	1x9 Gy	2.4	11.5	23		26	47	23.0;64.4;28.7
(21]	46, ALL	1x9 Gy	2.4	11.5	31		14	46	23.0;64.4;28.7
(30]	67	11x1.2 Gy	10.2	14.7	30	42	13.4	45	17.7;34.2;19.5
(33]	100	6x2 Gy	1.3 and 1.6	13.4	34	57.9		53.3	17.5;35.5;19.7
(41]	184	1x10 Gy	2.18	12.6	22.9	48.4	36.4		25.8;71.0;32.1

[a] assuming no shielding of organs; [b]mean value of two regimens

Overall survival was increased in five studies (11,14,20,28,36) and decreased in two studies (4,7). The pooled overall survival was significantly higher for the high-BED schemes. Figure 1 summarizes the above findings.

Dose-Effect Relationships

Dose-effect relationships were constructed for the four endpoints, using the dataset of Table 1. In figure 2A, the relapse incidence decreases with increasing BED values. The slope of the curve, weighted for the number of patients per study, is significantly different from 0 ($p = 0.035$).

Table 2. Relapse incidence, disease-free survival, treatment-related mortality, and overall survival in the three randomized trials (7,14,20) and four studies (4,11,28,36) comparing two or three TBI schemes at low and high BED, relative risks, and pooled relative risk. BED: biologically effective dose; N: total number of patients; n: number of responders; NS: nonsignificant (95%CI includes value 1); RR (95%CI): relative risk and 95% confidence limits; S: significant; TBI: total-body irradiation. The low-BED group numbers in the study of Bieri et al. (4) contributed once to the pooled values.

Reference	BED (Gy) Low	n/N Low	BED (Gy) high	n/N high	RR (95%CI)	Significance
A. *Relapse incidence*						
(4]	11.6	6/24	14.2	10/66	0.61(0.25-1.49)	NS
(4]	11.6	6/24	16.2	10/26	1.54(0.66-3.59)	NS
(7]	13.9	12/34	18.5	4/37	0.31(0.11-0.86)	S
(11]	12.1	16/53	13.9	6/45	0.43(0.18-1.01)	NS
(14]	13.4	6/27	13.8	3/26	0.52(0.14-1.86)	NS
(20]	13.1	20/73	16.8	11/74	0.54(0.28-1.05)	NS
(28]	12.0	7/36	15.1	5/48	0.54(0.19-1.55)	NS
(36]	12.8	19/65	13.9	28/106	0.90(0.56-1.48)	NS
Pooled values		86/312 (28%)		77/429 (18%)	0.65(0.50-0.85)	S
B. *Disease-free survival*						
(4]	11.6	10/24	14.2	33/66	1.20(0.71-2.04)	NS
(4]	11.6	10/24	16.2	10/26	0.92(0.47-1.82)	NS
(7]	13.9	20/34	18.5	22/37	1.01(0.69-1.49)	NS
(28]	12.0	20/36	15.1	33/48	1.24(0.87-1.75)	NS
(36]	12.8	28/65	13.9	59/106	1.29(0.93-1.79)	NS
Pooled values		78/159 (49%)		157/283 (55%)	1.13(0.94-1.37)	NS
C. *Treatment-related mortality*						
(4]	11.6	5/24	14.2	19/66	1.38(0.58-3.29)	NS
(4]	11.6	5/24	16.2	5/26	0.92(0.30-2.80)	NS
(7]	13.9	4/34	18.5	12/37	2.76(0.98-7.73)	NS
(11]	12.1	22/53	13.9	14/46	0.73(0.43-1.26)	NS
(14]	13.4	15/27	13.8	8/26	0.55(0.28-1.08)	NS
(20]	13.1	24/73	16.8	26/74	1.07(0.68-1.68)	NS
(28]	12.0	11/36	15.1	11/48	0.75(0.37-1.53)	NS
(36]	12.8	25/65	13.9	25/106	0.61(0.39-0.97}	S
Pooled values		106/312 (34%)		120/429 (28%)	0.82(0.66-1.02)	NS
D. *Overall survival*						
(4]	11.6	15/24	14.2	37/66	0.90(0.62-1.31)	NS
(4]	11.6	15/24	16.2	12/26	0.74(0.44-1.24)	NS
(7]	13.9	22/34	18.5	22/37	0.92(0.64-1.32)	NS
(11]	12.1	27/53	13.9	31/46	1.32(0.95-1.84)	NS
(14]	13.4	8/27	13.8	14/26	1.74(0.91-3.32)	NS
(20]	13.1	28/73	16.8	33/74	1.16(0.79-1.71)	NS
(28]	12.0	21/36	15.1	37/48	1.32(0.96-1.81)	NS
(36]	12.8	29/65	13.9	68/106	1.44(1.06-1.95)	S
Pooled values		150/312 (48%)		254/429 (59%)	1.23(1.07-1.42)	S

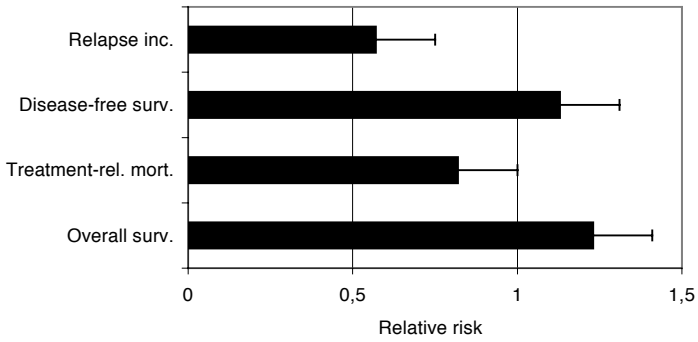


Figure 1. Pooled relative risks for relapse incidence, disease-free survival, treatment-related mortality, and overall survival at high biologically effective dose (BED) as compared to low BED, of the three randomized trials and four studies comparing two or three TBI schemes.

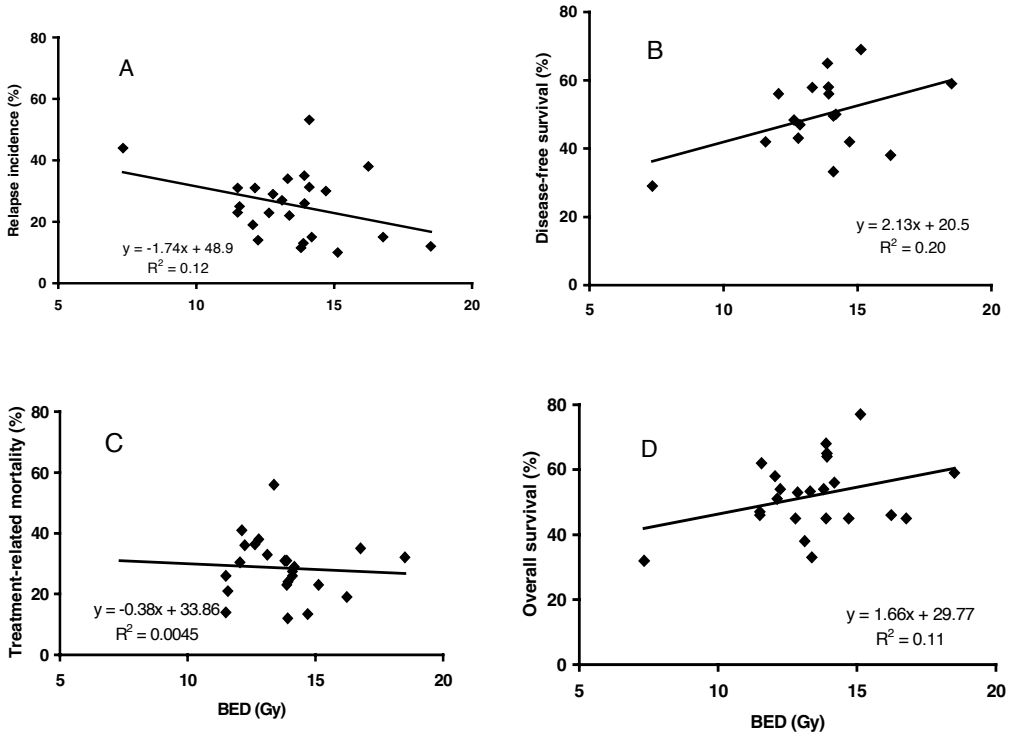
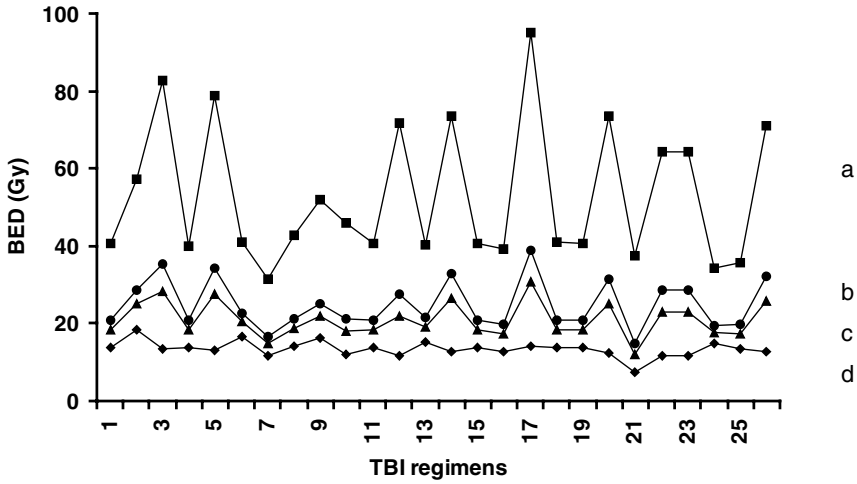


Figure 2a to 2d. Relapse incidence (a), disease-free survival (b), treatment-related mortality (c) and overall survival rate (d) as function of the biologically effective dose (BED) (α/β = 10 Gy and μ = 1.4/h).

In figure 2B, the slope of the disease-free survival curve, weighted for the number of patients per study, is significantly different from 1 (p = 0.02); disease-free survival increases with increasing BED.

In figures 2C and 2D, the treatment-related mortality rates and overall survival as function of BED are shown. No significant difference in treatment-related mortality as function of the BED is observed. The slope of the overall-survival curve, weighted for the number of patients per study, is significantly different from 1 (p = 0.03); overall survival increases with increasing BED



Figigure 3. Biologically effective doses (BED) for various TBI regimens calculated for eye lens, kidney, lung, and leukemic cells with appropriate α/β and μ values. The order in the TBI regimens on the horizontal axis is identical with the ranking in Table 1. a (squares): eye lens; b (circles): kidney; c (triangles): lung, and d (diamonds): leukemic cells.

L*ate Normal-Tissue Morbidity*

The BED values of the regimens in Table 1 for eye lens, lung, and kidney, supposing no shielding, are shown in Figure 3. In addition, the BED values for leukemia are shown. The TBI regimen numbers in Figure 3 correspond with the ranking in Table 1.

DISCUSSION

The role of high-dose TBI is still under debate. Gale et al. (19) stated that different TBI schemes all had the same results. Some authors reported a higher overall survival with

increasing TBI dose (28, 36], others found opposite results (4,7). Recently, totally different low-intensity conditioning regimens with low-dose TBI have extended the benefit of graft-versus-tumour effect to patients who are not candidates for fully ablative stem cell transplantations by virtue of their age or existing comorbidities (32,39). TBI schemes used, e.g., 1 x 2 Gy, are easy to apply, as shielding is not necessary and toxicity is low. In terms of leukemic cell kill they are, however, very suboptimal.

Vriesendorp et al. (45) stated that different TBI procedures could not be compared without radiobiological “normalization”. We performed “normalization” according to the LQ-BED model.

We restricted ourselves to the results of patients who suffered from AL, underwent high-dose TBI, and received allogeneic HSCT. AL is known to relapse predominantly within the first 2 years after HSCT (7,8). Studies were included, if the follow-up time was at least 3 years.

From the three randomized studies found in the literature no meaningful conclusions could be drawn, probably due to the relatively low number of patients per treatment arm. For the RR analysis we therefore decided to pool all available data. We observed a significantly lower relapse incidence, a higher disease-free survival, less treatment-related mortality, and a significantly higher overall survival for the high-BED treatments. The results were as expected, based on the assumption that at higher BEDs the leukemic cell kill is increased.

The dose-effect relationships for the four endpoints show the tendencies as mentioned before. Our analysis of the pooled data therefore indicates a benefit for higher BED values. A relatively high BED for leukemic cells can only be applied taking the tolerance BED of normal tissues into account (25,27). In most of the studies, the lung dose was restricted to a total dose of 8-9 Gy corresponding to a BED value for lung tissue of 12-14 Gy. In a number of publications it was recommended to limit the dose to the kidneys to 10-12 Gy in a fractionated regimen (5,23,24,29,31). This corresponds to a BED value of about 17 Gy (26). In Figure 3, the BED values for leukemia, lungs, kidneys, and eye lenses for the various TBI schemes, assuming no shielding of the organs (Table 1), are shown. It indicates that in the majority of the TBI treatments the kidney dose applied might have been too high in view of the recommended kidney dose limit. The TBI schemes with the highest kidney BED values are those using single doses (3, 14, 20). The incidence of severe cataracts for BED values of the lens tissue of less than about 40-45 Gy is negligible (44). In about half of the TBI treatments the “safe” lens dose is exceeded. The highest BED values for eye lens were found for single-dose regimens.

The requirements for an optimal TBI scheme therefore are: BED for leukemic cells as large as possible, i.e., BEDs of 15 Gy and larger, and BED for lungs, kidneys and eye lenses not exceeding 15 Gy, 17 Gy, and 45 Gy, respectively.

In Table 3, the TBI schemes of Table 1 are summarized. The TBI schemes with single doses (> 5 Gy) and almost all of the fractioned dose schedules should all be applied with shielding of the lungs, kidneys, and eyes. Only the scheme with 6 x 1.67 Gy can be applied without shielding. However, the $BED_{leukemia}$ is low (BED=11.6 Gy). The schemes with high $BED_{leukemia}$ values can only be applied with adequate shielding. The most widely used scheme in this survey is 6 x 2 Gy. Lung and kidney shielding (no eye shielding) has to be applied, the leukemic cell kill, however, is considered suboptimal (< 15 Gy).

Table 3. Summary of TBI regimens with requirements for minimum BED values for leukemia and maximum BED values for eye lens, kidney and lung. BED: biologically effective dose; NSh: no shielding of organ required; Sh: shielding of organ required; TBI: total-body irradiation; Y: BED value >15 Gy.

	1x5 Gy	1x7.5 Gy	1x9/9.2 Gy	1x10 Gy	3x3.3 Gy	5x2.2 Gy	6x1.67 Gy	6x2 Gy	6x2.25 Gy	7x2.25 Gy	8x1.65 Gy	11x1.2 Gy	11x1.35 Gy
BED leukemia >15 Gy								**Y**	**Y**	**Y**		**Y**	
BED Eye lens <45 Gy		**Sh**	**Sh**	**Sh**	**NSh**	**NSh**	**NSh**	**NSh**	**Sh**	**Sh**	**NSh**	**NSh**	**NSh**
BED kidney <17 Gy	**NSh**	**Sh**	**Sh**	**Sh**	**Sh**	**Sh**	**NSh**	**Sh**	**Sh**	**Sh**	**Sh**	**Sh**	**Sh**
BED lung <15 Gy	**NSh**	**Sh**	**Sh**	**Sh**	**Sh**	**Sh**	**NSh**	**Sh**	**Sh**	**Sh**	**Sh**	**Sh**	**Sh**

Shielding will not only result in a lower BED for the shielded organ, it also causes a lower BED for the leukemic cells and the bone marrow present in the tissues (e.g. ribs) in the shadow of the shielding blocks. The highly fractionated schemes must be considered to be the most effective. For example, with the scheme of 11 x 1.35 Gy, the $BED_{leukemia}$ is 16.8 Gy, and the BED_{kidney} is 22.8 Gy (Table 1). With shielding of the kidneys to 11 x 1.1 Gy

resulting in a BED_{kidney} of 17.4 Gy, the dose reduction is only 20% and the $BED_{leukemia}$ behind the shielding is still 13.3 Gy. For a single-dose treatment, e.g., 1 x 10 Gy (Table 1), the $BED_{leukemia}$ is 13.1 Gy, and the BED_{kidney} is 34.4 Gy. The dose reduction must be 34% to 1 x 6.6 Gy resulting in a BED_{kidney} behind the shielding of 17.2 Gy; however, the $BED_{leukemia}$ behind the shielding is only 8 Gy

CONCLUSION

High BED values appear to cause less leukemia relapses and a higher disease-free and overall survival. With highly fractionated schemes a high $BED_{leukemia}$ can be obtained. Shielding of lungs and kidneys during TBI seems to be unavoidable. However, the measure of shielding in highly fractionated schemes is relatively limited as compared to hypofractionated or single-dose TBI schemes. The question posed by Bieri et al. (4) whether more dose is better in TBI, can be answered positively. Prospective trials, in which TBI schemes with multiple fractions are compared with a low number of fractions schemes, are warranted using the BED concept. Purpose should be to elucidate whether highly fractionated schemes with a high total dose indeed yield into better treatment results than single-dose regimens or regimens with a low number of fractions.

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Chapter 7

Chapter 8

RESULTS OF HEMATOLOGICAL STEM CELL TRANSPLANTATION AFTER TREATMENT WITH DIFFERENT HIGH-DOSE TOTAL- BODY IRRADIATION REGIMENS IN FIVE DUTCH CENTERS

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Chapter 8

ABSTRACT

Purpose: To evaluate results of high-dose total-body irradiation (TBI) regimens for hematological stem cell transplantation (HSCT).

Methods and materials: 1032 patients received high-dose TBI before autologous or allogeneic HSCT for acute leukemia (AL) and non-Hodgkin's lymphoma (NHL). TBI regimens were normalized using the biological effective dose (BED) concept. BED values were divided into three dose groups. Study endpoints were relapse incidence (RI), non-relapse mortality (NRM), relapse-free survival (RFS) and overall survival (OS). Multivariate analysis was performed, stratified by disease.

Results: In the highest dose group (BED > 18 Gy) the RI was significantly lower and the NRM higher *vs*. the lower dose groups. A significant influence on RFS and OS was not found. Relapses in the eye region were found after shielding to very low doses. Age was of significant influence on OS, RFS and NRM in favor of younger patients. NRM of patients >40 years was significantly increased, OS was decreased. There was no influence of age on RI. Male patients had a better OS and RFS and a lower NRM. Type of transplantation significantly influenced RI and NRM, for AL as well as NHL. There was no influence on RFS and OS.

Conclusion: RI and NRM are significantly influenced by the TBI regimen, OS and RFS are not. Age was of highly significant influence on NRM, there was no influence of age on RI. An adverse influence of shielding could not be proven. Hyperfractionated TBI with a high BED might be useful assuming the NRM can be reduced.

INTRODUCTION

Hematological stem cell transplantation (HSCT) for acute leukemia (AL) and non-Hodgkin's lymphoma (NHL) is often the only curative therapy (1). Improvements in patient care have led to an increasingly successful outcome (2-4). High-dose total-body irradiation (TBI) is reported to be important in the conditioning before HSCT (5-7). In practice, every center has its own TBI regimen and technique, including the application of shielding blocks. As TBI may influence the outcome after HSCT, it is important to evaluate the effectiveness and toxicity of the various TBI treatments. We evaluated the results of autologous and allogeneic HSCT after high-dose conditioning in 1032 patients, and compared the outcome for the endpoints relapse incidence (RI), non-relapse mortality (NRM), relapse-free survival (RFS) and overall survival (OS). Attention was also paid to the effect of dose reduction in the shadow of the shielding blocks on RI.

METHODS AND MATERIAL

Patients

All 1032 patients (137 children, 895 adults) had high-dose TBI as part of their conditioning regimen before autologous (n = 228) or allogeneic (n = 804) HSCT for AL (n = 790) and NHL (n = 242). They were treated from 01-01-1990 until 01-01-2002, in five university medical centers in The Netherlands. The analysis was based on data derived from the files of the Netherlands stem cell transplantation registry (TYPHON). To complete the files and record accurately sites of relapse, all patient files were examined personally (M.L. van Kempen-Harteveld). Patients with a reduced intensity-conditioning (RIC) regimen or with chemotherapy alone were excluded. Data collecting started two years after 01-01-2002. Patient characteristics, age, gender, diagnosis, type of HSCT and remission status, are listed in Table 1. Tumor responses of the non-Hodgkin lymphomas were classified according to the International Workshop Criteria (8).

Source of stem cells, preparative regimen and graft-versus-host disease (GVHD) prevention

In 76.7% of the patients the graft was derived from bone marrow cells, in 22.7% from peripheral blood stem cells and in 0.6% from cord blood. T-cell depletion by *ex vivo* manipulation of cells was performed in 85% (n = 682) of allogeneic transplanted patients.

Table 1. Patient characteristics

		AL	NHL	Total
Age (year)	Mean	31.9 (range 2.3 – 64.1)	41.9 (range 15.7- 64.9)	34.24 (range 2.3 – 64.9)
Gender	Male	446 (43.2%)	144 (14.0%)	590 (57.2%)
	Female	344 (33.3%)	98 (9.5%)	442 (42.8%)
	Total	790 (76.5%)	242 (23.5%)	1032 (100%)
Diagnosis	ALL	325		325 (31.5%)
	AML	465		465 (45.1%)
	NHL			
	Low mal.		76	76 (7.4%)
	Interm. mal.		35	35 (3.4%)
	High mal.		126	126 (12.1%)
	Unknown		5	5 (0.5%)
	Total	790 (76.5%)	242 (23.5%)	1032 (100%)
Type of HSCT	Autologous	121 (11.7%)	107 (10.4%)	228 (22.1%)
	Allogeneic	669 (64.8%)	135 (13.1%)	804 (77.9%)
	Total	790 (76.5%)	242 (23.5%)	1032 (100%)
Remiss. Status	CR	733 (71.0%)	73 (7.1%)	806 (78.1%)
	PR	2 (0.2%)	147 (14.2%)	149 (14.4%)
	NR	53 (5.1%)	22 (2.1%)	75 (7.3%)
	Unknown	2 (0.2%)	0 (0%)	2 (0.2%)
	Total	790 (76.5%)	242 (23.5%)	1032 (100%)

Abbreviations: AL = acute leukemia; NHL = non-Hodgkin's lymphoma; ALL = acute lymphatic leukemia; AML = acute myeloid leukemia; Low mal. = low malignancy; Interm. mal. = intermediate malignancy; High mal. = high malignancy; HSCT = hematological stem cell transplantation; Remiss. status = remission status; CR = complete remission; PR = partial remission; NR = no remission.

Chemotherapy used for conditioning is listed in Table 2.

GVHD prophylaxis consisted of cyclosporine (n = 553), cyclosporine and methotrexate (MTX) (n = 111), cyclosporine and corticosteroids (n = 7) and MTX and steroids (n = 2). Of the patients who received a T-cell depleted allogeneic transplantation 131 (16.3%) did not receive drugs for GVHD prevention

Table 2. Chemotherapy within the conditioning regimen

	Drugs	Dose	Number of patients
One drug	Cy	120 mg/kg	n = 653 (63.3 %)
	Other		n = 2 (0.2 %)
Two drugs	Cy + Idarubicine	120 mg/kg + 42 mg/m^2	n = 189 (18.3 %)
	Cy + Etoposide	120 mg/kg + 700mg/m^2	n = 125 (12.1 %)
	Cy + ARA-C	120 mg/kg + 4 g/m2	n = 60 (5.8 %)
	Other		n = 2 (0.2 %)
Three drugs	Cy + Etoposide + ARA-C		n =1 (0.1 %)

Abbreviations: Cy = cyclophosphamide; ARA-C = Cytarabine.

TBI

TBI was administered with a linear accelerator, with energies varying from 5 to 23 MV. The TBI dose was administered in one or two fractions and varied between 6 and 12 Gy. Children <10 years of age (n = 78) were treated with a single TBI dose of 6 – 7.5 Gy. Dose rates were in the range of 0.075 - 0.18 Gy/min (Table 3).

Of the patients 538 received TBI by anterior and posterior (AP/PA) parallel opposing fields in a lying position, 237 by AP/PA opposing fields in a sitting position and 257 by lateral opposing fields in a sitting position.

Lead containing blocks shielding the lungs in order to prevent radiation pneumonitis were applied to all patients, except 73 children, < 10 years, treated with TBI doses of 6 – 7.5 Gy (Table 3).

Shielding of the kidneys was used in 354 patients and blocks to protect eye lenses in 809 patients. Blocks used in centers shielding eyes in the AP field were 3 cm Ø. When shielding was applied in the AP field, the shadow of the blocks resulted in two tiny cylinders of reduced dose intensity into the brain.

Characteristics of the TBI, including dose on leukemic and NHL cells in the shadow of shielding blocks are listed in Table 3.

Tabel 3. Characteristics of the Total Body Irradiation (TBI)

Number of patients	Technique TBI	Dose Rate Overall (Gy/min)	TBI (Gy)	BED_L TBI (Gy)	Eye Shield.	Reg. Eye (Gy)	BED_L Eye (Gy)	Lung Shield.	Reg. Lung (Gy)	BED_L Lung (Gy)	Kidney Shield.	Reg. kidney (Gy)	BED_L kidney (Gy)
2	AP/PA l	0.180	1 x 6	9.29	+	1 x 3.5	4.62	-	1 x 6	9.29	-	1 x 6	9.29
12	AP/PA 1	0.180	1 x 7	11.41	+	1 x 3.75	5.02	-	1 x 7	11.41	-	1 x 7	11.41
5	Lateral s	0.075	1 x 7.5	11.84	+	1 x 3.75	4.84	+	1 x 6.5	9.76	+	1 x 6.5	9.79
3	AP/PA 1	0.150	1 x 7.5	12.42	+	1 x 4.5	6.27	-	1 x 7.5	12.42	-	1 x 7.5	12.42
198	Lateral s	0.075	2 x 4.5	12.45	+	2 x 2.25	5.36	+	2 x 4	10.73	-	2 x 4.5	12.45
2	AP/PA 1	0.180	1 x 7.5	12.53	-	1 x 7.5	12.53	-	1 x 7.5	12.53	-	1 x 7.5	12.53
54	AP/PA 1	0.180	1 x 7.5	12.53	+	1 x 4	5.43	-	1 x 7.5	12.53	-	1 x 7.5	12.53
8	Lateral s	0.075	1 x 8	12.86	+	1 x 4	5.22	+	1 x 6	8.73	+	1 x 6	8.73
4	AP/PA 1	0.100	1 x 8	13.19	-	1 x 8	13.19	+	1 x 7	10.97	-	1 x 8	13.19
37	AP/PA s	0.150	1 x 8	13.55	-	1 x 8	13.55	+	1 x 7	11.25	-	1 x 8	13.55
8	AP/PA l	0.180	1 x 8	13.68	+	1 x 4	5.42	+	1 x 6	9.19	-	1 x 8	13.68
39	AP/PA 1	0.100	2 x 5	14.37	-	2 x 5	14.37	+	2 x 4.25	11.66	-	2 x 5	14.37
29	AP/PA s	0.150	1 x 9	15.90	+	1 x 8	13.45	+	1 x 7	11.48	-	1 x 9	15.90
166	AP/PA 1	0.180	1 x 9	16.08	+	1 x 5	7.19	+	1 x 6	9.15	-	1 x 9	16.08
46	Lateral s	0.075	2 x 6	17.83	+	2 x 3	7.46	+	2 x 4	10.59	+	2 x 4	10.59
148	AP/PA 1	0.100	2 x 6	18.14	-	2 x 6	18.14	+	2 x 4.25	11.58	+	2 x 5	14.26
177	AP/PA s	0.150	2 x 6	18.46	+	2 x 5	14.49	+	2 x 4.25	11.75	+	2 x 5	14.49
29	AP/PA 1	0.130	2 x 6	18.36	+	2 x 3.3	8.52	+	2 x 4	10.83	-	2 x 6	18.36
65	AP/PA 1	0.180	2 x 6	18.58	+	2 x 3.3	8.59	+	2 x 4.25	11.80	-	2 x 6	18.35

Abbreviations: AP/PA = antero-posterior opposing irradiation fields; l = patient in lying position; Lateral = lateral irradiation fields; s = patient in sitting position; Gy = gray; Reg. = regimen; BED_L = Biologically Effective Dose for leukemic cells; The horizontal lines divide the 3 BED/TBI groups used for analysis.

Linear-quadratic and biological effective dose (LQ-BED) concept

Occurrence of a biological effect (E) of irradiation depends on the radiation dose in a linear and quadratic way (LQ): $E = n (\alpha d + \beta d^2)$, where d is dose per fraction, n number of fractions and α en β tissue specific constants.

From that equation, the biological effective dose (BED) can be derived (9-11)

$$BED = nd [1 + d /(\alpha/\beta)]$$

For fractionated low-dose-rate irradiation

$$BED = nRT (1 + kR/(\alpha/\beta)]$$

where R is the dose rate, T treatment time per fraction, and

$$k = 2 (1 - \{1 - \exp (-\mu T)\}/(\mu T)] / \mu.$$

Factor k depends on sublethal damage repair rate μ during low-dose-rate irradiation and treatment time T. Parameter μ is related to the half-time for monoexponential repair of sublethal damage T1/2, where T1/2 = ln2/ μ (13).

The value of α/β for acute reacting tissues like leukemia cells is about 10 Gy and about 2 – 3 Gy for late reacting tissues. We used for the endpoints mentioned, α/β = 10 Gy and μ = 1.4/h (10,11).

Shielding results into lower BED values for malignant target cells in the shadow of the blocks (Table 3).

BED values of the various TBI regimens were divided into three groups: I. BED ≤ 14 Gy (n = 337), II. 14 < BED ≤ 18 Gy (n = 278) and III. BED > 18 Gy (n = 417).

Site of relapse

With respect to the shadow of lung blocks are concerned: relapses in lungs, pericardium, pleura, breasts and also, in case of lateral TBI field, mediastinal nodes. In the shadow of kidney blocks are of importance: relapses in kidneys, spleen and liver, in the shadow of eye blocks: relapses in eyes and tissue surrounding the eyes and localized in the CNS. The latter only when an AP/PA TBI technique is used. When there was doubt about the origin of a lesion - caused by relapse or any other cause the lesion was documented as 'of unknown origin'.

Statistical analysis

Primary endpoints were NRM, RI, RFS and OS. NRM was defined as death during complete remission from causes related to the transplant procedure. Relapse was defined

as persistent or recurrent disease. For analyses of RFS, relapses or death from any cause were considered as events. For analyses of OS, events were death from any cause.
Univariate probabilities of RFS and OS were calculated using Kaplan-Meier analysis; the log-rank test was used for univariate comparisons of the effect of the variable risk factors on OS, RFS, RI and NRM. Cox proportional hazards regression analysis was applied, stratifying for disease, to assess the independent significance of the variables on the endpoints of the study. Variables considered in multivariate analysis were type of transplant, age group, gender and BED/TBI group. For all estimates, a competing-risk analysis was performed producing estimates for the cumulative incidence of events. Relapse was used as competing risk for death and *vice versa* (hence RI and NRM are estimated simultaneously in one model). Ninety-five percent confidence intervals (95% CI) for all probabilities and P values of pair-wise comparisons were derived from point-wise estimates and were calculated using standard techniques.

RESULTS

Cumulative survival of all patients was 0.56 (standard error, S.E., = 0.02) at 24 month, 0.49 (S.E. = 0.02) at 48 month, and 0.43 (S.E. = 0.02) at 10 years.

Influence of the TBI regimen

In univariate Cox model, no significant influence of BED/TBI group on OS and RFS was found. There was, however, a significant influence on RI and NRM: the group with the highest BED had a significantly lesser probability to develop a relapse than lower BED regimens ($p = 0.004$), however, the NRM was significantly higher ($p = 0.001$) (Table 4).
Results of a competing-risk analysis quantifying influence of the three BED groups are shown in Fig.1. The cumulative incidence of all events appeared to be not very different for all three BED groups (at 24 month: 0.44, 0.53 and 0.51 respectively, at 10 years: 0.57, 0.62 and 0.58 respectively). RI, however, is considerably lower in the highest BED group, while NRM for this group is higher. Cumulative incidences of relapse at 24 month of the three BED groups I, II and III are 0.32, 0.39 and 0.25, respectively. Cumulative incidences of NRM at 24 month are 0.12, 0.14 and 0.26, respectively (Table 4, Fig. 1).
Cox regression analysis was performed to assess in multivariate analysis the risk factors and find out whether the variables were independently significant. Stratification was

applied by disease. Patients in BED/TBI group III (> 18 Gy) had a hazard ratio (HR) to develop a relapse of 0.78 (95% CI = 0.60 – 1.01) (p = 0.05) *vs.* patients with BED/TBI group I (≤ 14 Gy). HR for NRM of patients with the highest BED/TBI group was 1.74 (95% CI = 1.25 – 2.44) (p = 0.001) *vs.* patients with the lowest BED/TBI group.

To test whether the effects of the dose groups were dependent on the other variables, interaction tests were applied. There was no indication that the effect of the dose group was dependent on gender and age of the patient or the transplantation type (interaction tests all with p > 0.20).

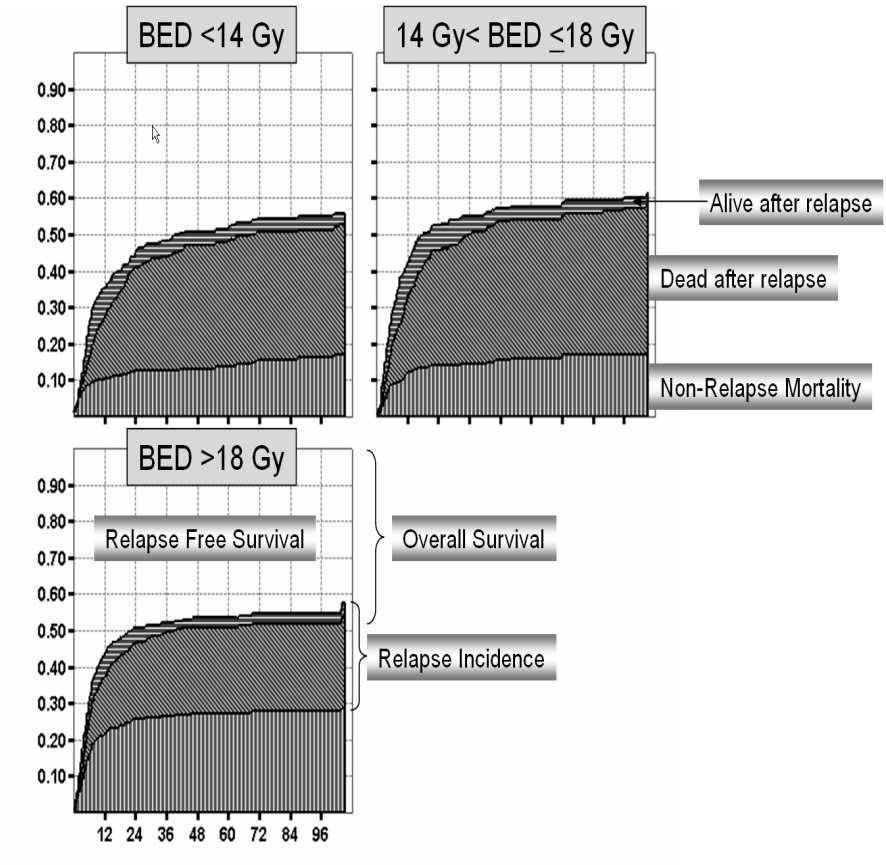

Figure 1. Cumulative incidence of events according to biological effective dose (BED/TBI) group as a function of time after hematological stem cell transplantation (HSCT).

Table 4. Influence of type of disease, type of transplant, gender and BED/TBI group in compared-risk analysis

	OS (%)		R.I (%)		NRM (%)		RFS (%)		Dead after R (%)		Alive after (%)	
	24 m	72 m	24 m	72 m	24 m	72 m	24 m	72 m	24 m	72 m	24 m	72 m
AL	53	45	33	37	18	20	49	43	29	35	4	1
NHL	64	57	24	29	19	23	58	48	17	20	9	7
	(p = 0.01)		(p = 0.09)		(n.s.)		(n.s.)					
Autologous	60	48	41	47	6	11	53	43	33	41	7	6
Allogeneic	54	47	28	31	22	24	50	45	24	29	4	3
	(n.s.)		(p = 0.05)		(p = 0.001)		(n.s.)					
Male	57	50	34	33	17	19	53	48	26	31	4	3
Female	53	44	32	37	21	23	47	40	26	33	6	4
	(p = 0.04)		(n.s.)		(p = 0.04)		(p = 0.02)					
Age < 20 years	55	52	33	35	13	13	54	52	31	35	2	1
Age ≥ 20 – 40 years	58	48	31	35	16	19	53	46	26	33	5	2
Age ≥ 40 years	53	44	30	35	23	27	47	38	24	29	6	6
	(p = 0.06)		(p = 0.0549)		(p = 0.001)		(0.01)					
BED_L ≤ 14 Gy	59	49	32	39	12	16	56	45	28	35	4	4
14< BED_L ≤ 8 Gy	54	45	39	42	14	17	47	42	31	38	7	4
BED_L >18 Gy	53	48	25	27	26	28	49	45	21	24	4	3
	(ns)		(p = 0.004)		(p = 0.001)		(n.s.)					

Abbreviations: BED_L = biological effective dose for leukemic cells; TBI = total body irradiation; OS = survival; RI = relapse incidence; NRM = non-relapse mortality; RFS = relapse free survival; m = month after HSCT; AL = acute leukaemia; NHL = non-Hodgkin's lymphoma; Gy = gray; R= relapse.

Influence of age, gender, type of transplantation and type of disease

Age

Patients were divided into three groups: I. < 20 years (n = 214), II. ≥ 20 – < 40 years (n = 394) and III. ≥ 40 years (n = 424). In univariate analysis, age was found to be of significant influence on NRM (p = 0.001) and RFS (p = 0.01) in favor of younger age. There was no

influence on RI (p = 0.90) (Table 4). Results of a competing risk analysis according to age, for all patients in the study, are shown in Fig. 2.

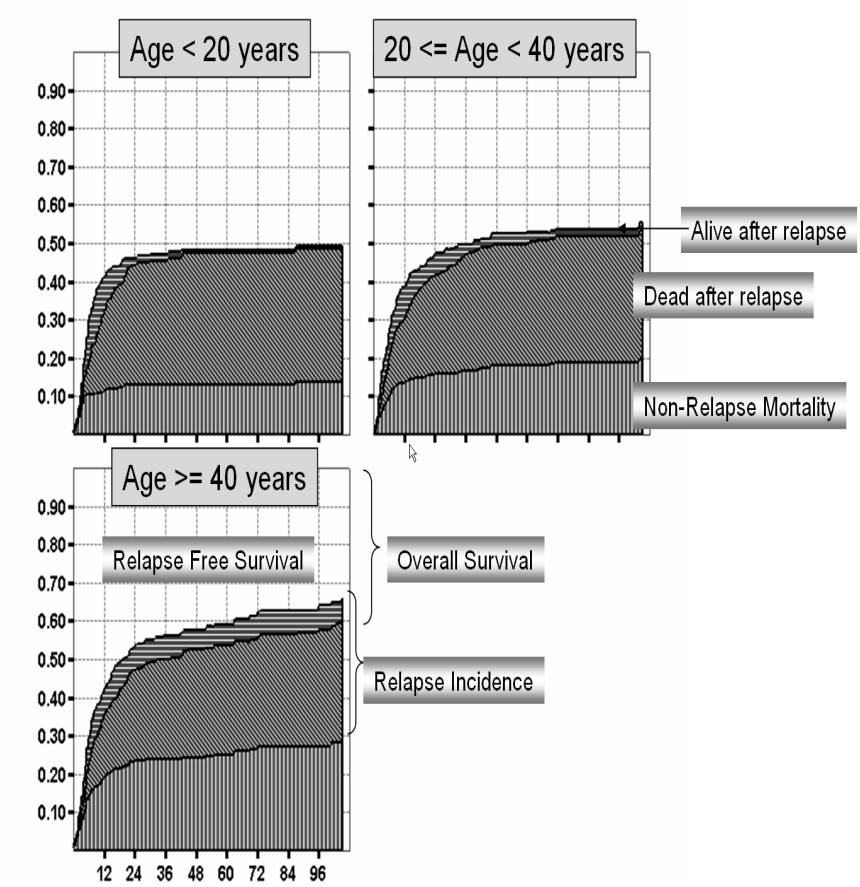

Figure 2. Cumulative incidence of transplant-related mortality (NRM), relapse incidence (RI) and overall survival (OS) as a function of time after hematological stem cell transplantation (HSCT) according to age group.

In Cox regression analysis, age group appeared to be an independently significant variable (assumed equal for both diseases since no interaction with disease was present). HR for NRM for patients ≥ 40 years was 2.28 (95% CI = 1.5 – 3.5) (p = 0.001) *vs.* patients < 20 years. Patients ≥ 40 years had a HR for OS that was 1.34 (95% CI = 1.05 - 1.71) (p =

0.02) vs. patients < 20 years and a HR for RFS that was 1.37 (95% CI = 1.08 – 1.73) (p = 0.01) *vs*. patients ≥ 20 years.

Gender

Gender appeared, in univariate Cox model, to be of significant influence on OS (p = 0.04) as well as RFS (p = 0.02), in favor of male patients (HR around 1.2). There was also a significant influence of gender on NRM (p = 0.04) but not on RI. In competing risk analysis, there were, apart from effects found in the univariate analysis, no major differences between male and female patients for mortality rate after relapse, and patients alive after relapse (Table 4).

In Cox regression analysis, gender appeared to be an independently significant variable for both diseases.

Transplantation type

Transplantation type was in univariate Cox model only of significant influence on RI and NRM. Allogeneic transplanted patients, AL as well as NHL, had a significantly lower probability of relapse (p = 0.001 and p = 0.05, respectively) and a higher NRM (p < 0.01 and p = 0.001, respectively) than autologous patients. No significant influence of transplantation type on OS and RFS could be demonstrated. In Cox regression analysis, stratified for disease, HR for NRM of allogeneic *vs.* autologous patients was 2.43 (95% CI = 1.55 – 3.8) (p = 0.001). Allogeneic patients had a HR to develop a relapse of 0.68 (95% CI = 0.53 – 0.88) (p = 0.01) *vs.* autologous patients.

Disease

In univariate Cox model patients with NHL had a significantly better OS (p < 0.01) and lower cumulative probability of relapse (p = 0.09) than patients with AL. In competing-risk analysis, NRM and RFS were not significantly different between NHL and AL patients, the higher OS of NHL patients appeared to be caused by a lower RI and a lower mortality rate once a relapse had occurred (Table 4).

Results of competing-risk analysis, quantifying the influence of transplantation type on the cumulative incidence of events - RI and NRM – for AL and NHL are shown in Fig. 3. Cumulative influence of all events was not significantly different for both groups; in allogeneic patients the RI was, however, considerably lower, while NRM was higher. Especially in allogeneic patients with NHL, the NRM appears to be relatively high.

In all models, interaction between type of disease and transplantation type was non-significant ($p > 0.20$), hence the effect of type of transplant may be assumed to be the same in AL and NHL for all outcome measures. This effect was estimated in a stratified Cox model (stratified by disease to allow for the non-proportional baseline curves of the two diseases).

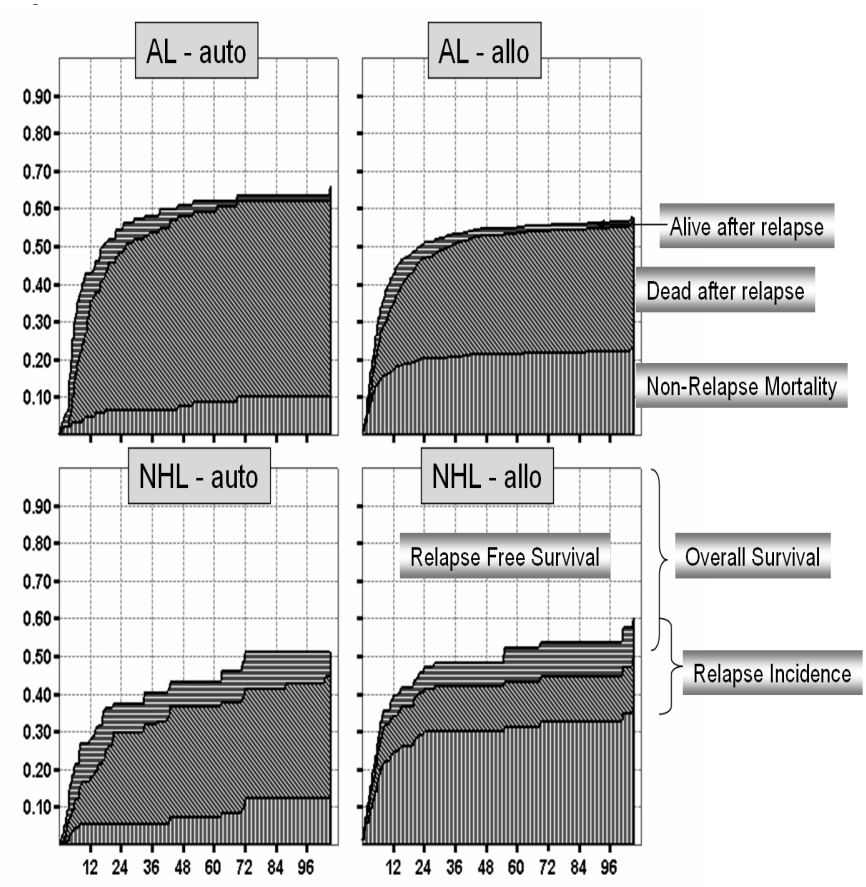

Figure 3. Cumulative incidence of events according to transplantation type for patients with acute leukemia (AL) and non-Hodgkin's Lymphoma (NHL) separately, as a function of time after hematological stem cell transplantation (HSCT).

Shielding blocks and site of relapse

A relapse developed in 357 patients. Fifty-nine of these had a relapse in ≥ two sites. Of the 60 extra-medullar (EM) / extra-nodal (EN) relapses, 11 were solitary (Table 5).

Table 5. Sites of first (solitary or combined) relapse after HSCT in 357 patients.

Site	AL	% of tot	NHL	% of tot	Total
Bone marrow	**275**	**77**	**20**	**5.6**	**295**
Lymph nodes above diaphragm	**1**	**0.3**	**37**	**10.4**	**38**
Lymph nodes below diaphragm	**2**	**0.6**	**27**	**7.6**	**29**
CNS	**16**	**4.5**	**4**	**1.1**	**20**
Eyes/ Eye lids/ Lacrim. glands (eye block)	**2**	**0.6**	**2**	**0.6**	**4**
Lungs/ Pleura/ Peric./ Breasts (lung block)	**2**	**0.6**	**4**	**1.1**	**6**
Liver/ Spleen / Kidneys (kidney block)	**0**	**0**	**7**	**2.0**	**7**
Other EN/ EM sites	**17**	**4.8**	**6**	**1.7**	**23**
Unspecified / Unknown site	**4**	**1.1**	**3**	**0.8**	**7**

AL = acute leukemia; NHL = non-Hodgkin's lymphoma; tot = total number; CNS = central nervous system; Lacrim. glands = lacrimal glands; Peric. = pericardium; EN / EM sites = extra nodal and extra medullar sites.

Lungs

Nearly all patients had lung blocks, apart from children with single doses ≤ 7.5 Gy. There was relatively little variation in BED values for leukemic cells in the shadow of the lung blocks (BED_L/TBI) (Table 3).

Local relapses in the region of the lungs, with and without shielding blocks, with accessory TBI regimens are listed in Table 6. Only one of those (a mediastinal nodal relapse) was solitary. All relapses except one happened in the shadow of a lung block. All occurred at relatively low BED value (i.e. < 14 Gy). Relapses in the mediastinum happened not more frequent in lateral field TBI technique compared antero-posterior irradiation: 6 (2.3%) lateral irradiated patients compared to 32 (4.5%) AP/PA irradiated patients.

Table 6. Biologically effective dose for leukemic cells (BED_L) at the relapse site in the shadow of the shielding blocks.

n relapses lung region	BED_L/lung (range, 8.73 – 12.53 Gy)	Regimen Lung (Gy)	Block	Accessory BEDL/TBI (Gy)	Regimen TBI (Gy)
1	9.15	1 x 6	+	16.08	1 x 9
8	10.73	2 x 4	+	12.45	2 x 4.5
2	11.25	1 x 7	+	13.55	1 x 8
1	11.41	1 x 7	-	11.41	1 x 7
7					
n relapses kidney region	**BED_L/kidney (range, 8.73 – 18.36 Gy)**	**Regimen Kidney (Gy)**	**Block**	**Accessory BED_L/TBI (Gy)**	**Regimen TBI (Gy)**
1	12.45	2 x 4.5	-	12.45	2 x 4.5
2	13.55	1 x 8	-	13.55	1 x 8
2	14.26	2 x 5	+	18.14	2 x 6
1	15.90	1 x 9	-	15.90	1 x 9
1	16.08	1 x 9	-	16.08	1 x 9
7					
n relapses eye region	**BED_L/eye (range, 4.62 – 18.14 Gy)**	**Regimen Eye**	**Block**	**Accessory BED_L/TBI (Gy)**	**Regimen TBI (Gy)**
2	5.36	2 x 2.25	+	12.45	2 x 4.5
1	5.43	1 x 4	+	12.53	1 x 7.5
1	7.46	2 x 3	+	17.83	2 x 6
4					
n relapses CNS	**BED_L shadow eye block in CNS (BED/eye) (Gy)**	**Regimen shadow (Gy)**	**Block**	**Accessory BED_L/TBI (Gy)**	**Regimen TBI (Gy)**
2	5.02	1 x 3.75	+	11.41	1 x 7
2	5.43	1 x 4	+	12.53	1 x 7.5
4	7.19	1 x 5	+	16.08	1 x 9
3	12.45	2 x 4.5	l.f.	12.45	2 x 4.5
1	13.45	1 x 8	-	15.90	1 x 9
1	14.37	2 x 5	+	14.37	2 x 5
2	14.49	2 x 5	-	18.46	2 x 6
5	18.14	2 x 6		18.14	2 x 6
20					

n = number; BED_L = biologically effective dose; Gy = gray; TBI = total body irradiation; l.f. = lateral field TBI technique; CNS = central nervous system.

Kidneys

Blocks to protect the kidneys were applied in 354 patients. Dose reduction was relatively limited. Only 34% of the patients had kidney shielding (Table 3). There were no solitary relapses in the kidney region. Only two of them developed behind a shielding block. No significant disadvantage for a low BED value of leukemic cells in the kidney region could be demonstrated.

Eyes

Eye blocks were applied in 809 patients. Dose reductions varied largely (Table 3). RI in the eye regions could be compared for a BED value for leukemic cells in the range of 4.62 to 18.14 Gy.

Four relapses occurred, all in the shadow of an eye block: two in the eyes, two in the eyelids (one in a patient with NHL, one with AL). Three relapses were solitary, including the two in an eyelid. Strikingly, all four relapses occurred behind shielding blocks reducing the BED to less than 7.5 Gy (Table 6).

CNS

Shielding of eye lenses using an AP/PA technique causes cylinders of decreased dose intensity into the brain. In total, 20 recurrences in the CNS were found: 9 (2.1%) in patients without areas of dose reduction in the CNS, 11 (2.0%) in patients with eye shielding AP/PA (Table 6). No increase of CNS recurrences as a result of AP/PA eye shielding technique could be demonstrated.

DISCUSSION

TBI regimen

Previously we found that higher BED/TBI doses were accompanied by a lower RI, no higher NRM and a better OS (12). In the present study we could only partly confirm this. We did find a lower RI in the TBI group with the highest BED, but also a higher NRM, for both diseases. The OS of the highest dosed TBI regimen was not significantly different from that of TBI regimens with the lowest BED values (Fig. 1, Table 4). In interaction tests there was no indication that the effect of the dose group was dependent on age, gender, or transplantation type.

Contrasting results like these are also reported in other studies (13,14). Some authors did find better results for higher TBI doses, however, often in specific groups of patients. Girinsky *et al*. reported significantly better results with a high-dosed *vs*. a lower-dosed TBI regimen, although only significant in autologous transplanted patients (15). Corvo *et al*. found better results with the higher-dosed TBI regimen for patients in first remission of ALL, comparing two TBI regimens (16). It should be noted that the results reported in our previous study were based on studies with, in the majority, multi-fractionated TBI regimens. Also, when single dose (SD) regimens were applied, the dose rates were distinctly lower than in the Dutch regimens, which contained only SD or two-fractions TBI with relatively high dose rates. Although BEDs for leukemic cells in SD regimens as well as in multiple fractionated regimens might be equally high, toxicity for normal tissues differs. The BED for leukemic cells of a 2 x 6 Gy TBI regimen, for instance, is about 18 Gy; while the BED/TBI of a frequently used TBI scheme like 6 x 2 Gy is about 14 Gy. For late effects on normal tissues, however, BED values of the mentioned regimens are 36 Gy and 20 Gy, respectively.

We found a statistical significant difference for RI and NRM in the highest BED group *vs*. the lower-dosed groups (Table 4) and relatively little difference for these parameters

Table 7. Patients (n = 1032) per BED group and per center.

Group of patients	center	Number of patients	% of subtotal	% of total
BED ≤ 14 Gy	**A**	**78**	**23.4**	**7.6**
	B	**211**	**63.4**	**20.4**
	C	**40**	**12.0**	**3.9**
	D	**4**	**1.2**	**0.4**
	E	**0**	**0**	**0**
	Subtotal	**333**	**100**	
14 ≤ BED ≤ 18 Gy	**A**	**166**	**59.3**	**16.1**
	B	**46**	**16.4**	**4.5**
	C	**28**	**10.0**	**2.7**
	D	**40**	**14.3**	**3.9**
	E	**0**	**0**	**0**
	Subtotal	**280**	**100**	
BED ≥ 18 Gy	**A**	**65**	**15.5**	**6.3**
	B	**0**	**0**	**0**
	C	**177**	**42.2**	**17.2**
	D	**148**	**35.3**	**14.4**
	E	**29**	**6.9**	**2.8**
	Subtotal	**419**	**100**	
	Total	**1032**		**100**

Abbreviations: BED = biologically effective dose for leukemic cells; Gy = gray.

between the two lower-dosed groups. An explanation might be the somewhat different chemotherapy regimen of a relatively high number of patients in the lowest BED group (Table 2). Another explanation could be a phenomenon reported by Loberiza *et al*. (17) and Frassoni *et al*. (18): there might be a variation in results among centers. Although the three BED/TBI groups represented patients from all centers, the distribution is not quite equal (Table 7).

Shielding blocks

Only few authors reported the influence of shielding blocks on RI and/or OS. No influence of kidney shielding on OS was reported (19,20). Some found an increased RI when the lung dose was lowered (21), others an increased RFS when the lung dose increased (22). In our study, it was not possible to correlate the dose on shielded lungs and kidneys to OS or RI, although lung blocks shielded a considerable amount of tissue and, as a consequence, also leukemic cells. Cause was a low number of local relapses, with certainty, diagnosed in the shadow of shielding blocks. Many more lesions were found but excluded when there was doubt about the diagnosis (GVHD, infection or relapse). Furthermore, measure of shielding lungs, in general, showed little variation, although the BED of the TBI regimens differed largely.

The relapses occurring in the eye region indicate that, as expected, a too low dose results in a higher chance for local relapse. As two of the (solitary) relapses were not located inside the eyes, the assumption that the eye, as a sanctuary site, is more vulnerable for relapse may not be valid. The dose deposited behind the eye blocks, in three of the four patients with an eye relapse, was 2 x 2.25 Gy, which is about similar to the dose that is applied in reduced intensity conditioning (RIC) regimens.

Multiple-fractionated TBI allows reduction of the measure of shielding compared to SD or two-fractions TBI, and so decreasing of dose reduction by shielding. In view of the leukemic cell kill, fractionation will, therefore, be of major advantage. Apart from a lesser need for dose reduction, the sparing of radiosensitive tissue from late effects of irradiation, in general, will be superior. Notwithstanding these sparing effects, some shielding will remain inevitable, dependent on the used TBI scheme (12).

Age

The effect of age on outcome after HSCT, in favor of younger age groups as we found, is reported before (23-25), although mostly with regard to children compared to adults, or patients > sixty years compared to younger patients.

We found the effect of age on RFS to be a gradual decrease of RFS with increasing age, due to an increase of NRM (Fig. 2, Table 4). HR for NRM in patients ≥ 40 years was 2.29 vs. patients < 20 years. In multivariate analysis, the influence of age group on OS was independently significant. There was no influence on RI. Few studies found an association of risk of relapse post-transplant with age (30). Willemze *et al.*, however, recently reported a lower RI and better survival in older children compared to younger, associated with the higher-dosed TBI regimen older children received (26). Interestingly, we found the same: when modelling the effect of age to subjects below 20 years, comparing those > 10 years to those ≤ 10 years, stratifying by disease and adjusting for allogeneic/autologous transplantation, we found an almost 2-fold reduced RI among the category 10-20 years (HR = 0.6, 95% CI + (0.4-0.9], p = 0.02); however, when the TBI dose is added to the model, the effect of age, adjusted for TBI dose, almost disappears (HR = 0.9, p = 0.70).

Gender

For the independently significant effect of gender on OS, RFS and NRM in favor of male patients, we have no explanation. Gratwohl *et al.* reported an unexplained higher incidence of death from viral infections among female patients, which is in agreement with the higher NRM for female patients we found (2). Belkaçemi *et al.* found a better OS and RFS for male patients (although not significant) (27). In our study a larger group of patients was included. As a consequence, the effect of gender may have become significant.

Type of transplantation

For the variable transplantation type we found no significant difference in OS and RFS, for AL as well as NHL. Only RI and NRM differed largely (Table 4, Fig. 3). Many authors reported this outcome as well (28-30), although some found better results with autologous or allogeneic transplantation separately (31,32).

Type of disease

Differences in survival figures for type of disease in favor of NHL patients (Table 4, Fig. 3), have been reported (28,32). Studies describing the comparison of results of HSCT in AL and NHL patients are scarce, as a logical consequence of the fact that diseases are concerned with a different course, treated in different ways. Survival figures describing results of HSCT in general are pointing in the direction of better results for lymphoproliferative malignancies, compared to acute leukemia's (33,34).

In this study, the results of autologous and allogeneic HSCT after high-dose chemo- and radiotherapy are compared. In autologous HSCT the benefit results only from the antileukemic effect of high-dose conditioning therapy, in allogeneic HSCT there is supposed to be benefit from both, high-dose conditioning and the immunological power of graft-versus-tumor (GVT) effect. Nevertheless, the results of autologous and allogeneic HSCT appear to be not significantly different. Obviously, the toxicity of allogeneic HSCT offsets the advantage of the combination GVT effect and antileukemic potential of high-dose conditioning. In the last years, many reports were published about reduced intensity conditioning (RIC) before allogeneic HSCT (35-38). This approach is based on the strategy of attaining donor cell engraftment with immunosuppressive agents after non-myeloablative conditioning, and relies only on immunological mechanisms (36). The results are promising, as the toxicity is relatively low (38). When comparing results of allogeneic HSCT after RIC with high-dose conditioning therapy, patients treated with RIC showed decreased NRM and less acute GVHD, but a higher RI. RFS for high- and low-dose conditioning appeared to be not statistically different (37-39).
It appears, therefore, that at present there are three possible approaches, having all about the same outcome with respect to survival. As RIC is easy to administer and relatively low toxic, high expectations were raised and it was suggested to apply the policy also to younger patients without risk factors. Things are, however, probably more complicated. Most likely patients have to be selected for a certain treatment, although the criteria are not completely known yet (36). Presumably, there are patients who benefit most from HSCT after RIC, and patients that should be treated with high-dosed conditioning, as they really have advantage from both, the antileukemic potential of high-dose chemo- and radiotherapy and the GVT effect. These are, probably, also the patients that may benefit from a TBI dose with a high BED value. Willemze *et al*. found, for instance, better results of a TBI regimen with a high BED vs. low BED regimens in children transplanted for AL (26).

The survival and NRM differences we found according to age showed that NRM in the oldest patient group is considerably higher than in younger groups, but that RI hardly differs (Fig. 2). As after RIC the RI is reported to be higher than after myeloablative treatment (38), it seems not wise to submit young patients to a RIC regimen, especially as in the recent years little effect on RI (2-4) is reported, but a significant reduction of the NRM as a consequence of reduction in death from infections and better management of GVHD (2,3). Yet it is difficult to indicate a cut off point for age. As RIC is successful, older

patients should benefit from it. However, as many authors now agree (35-39), randomized trials should be conducted assessing the best treatment options: high- or low-dose conditioning, and the best TBI regimens for high-dose conditioning, comparing schemes with different regimens, taking into account their BEDs for leukemic cells and for organs at risk.

In conclusion, age and gender were the only variables that influenced OS significantly, independently from type of disease. TBI regimens significantly influenced RI and NRM, but did not influence OS and RFS after autologous and allogeneic HSCT. No significant influence of shielding blocks on RI could be found. To select patients for the best treatment options, randomized trials are needed. For comparing the results of HSCT of large groups of patients, it is important to minimize treatment differences, such as conditioning regimen. To minimize dose reductions by shielding, it is advisable to apply a hyperfractionated TBI regimen.

Chapter 8

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Chapter 9

SUMMARY, CONCLUSIONS, GENERAL DISCUSSION

AND IMPLICATIONS FOR THE FUTURE

Chapter 9

SUMMARY

Chapter 1 gives an introduction to hematological stem cell transplantation (HSCT) and total body irradiation (TBI), complications, late effects and outcome of the treatment and outlines the aim of the thesis consisting of 6 main questions:

1. What are the characteristics of cataract development after TBI, when applied in one or two fractions, as part of the conditioning for HSCT and what are the consequences of a radiation cataract with respect to the visual impairment.
2. Is there a risk involved in preventively shielding the eyes during TBI.
3. Is it possible to predict the incidence of cataract formation for a certain TBI regimen.
4. What is the tolerance dose for kidney tissue when TBI, is applied as part of the conditioning for HSCT.
5. What are the requirements for an optimal TBI regimen concerning a maximal leukemic cell kill, a maximal OS and minimal late effects on lungs, kidneys and eye lenses.
6. What are the results of HSCT/TBI in The Netherlands.

Chapter 2 describes the results of a prospective study on cataract formation in 93 patients treated with TBI, applied in one or two fractions, as part of the conditioning for autologous (n = 42) or allogeneic (n = 51) HSCT in the Department of Radiotherapy of the University Medical Centre in Utrecht. The patients were treated from 1982, when BMT started in Utrecht, until 1995. During the years cataracts were assessed by regular made slides of the eye lenses with a fundus camera, on the Department of Radiotherapy. After 1989 the photographer of the Department of Ophthalmology made the slides. For assessing and follow-up of the cataract formation, slides were made at every visit, at least yearly, whether an opafication was seen or not. To grade the cataracts, we used the classification system of Schipper (1,2). The system was based on photographs of posterior subcapsular radiation cataracts of the human lens and consisted of eight grades of severity (0/I-IV).
The probability to develop a cataract appeared to be 100%. Steroids administered for GVHD adversely influenced latency time and final severity of a cataract. In autologous transplanted patients a cataract developed after a median period of 58 month, in allogeneic patients after 46 month without and after 33 months with steroid treatment. Cataracts were found to stabilize ≤ 30 months after they had been observed for the first time. In autologous transplanted patients the incidence of stable high-grade cataract was 48%. In allogeneic patients, treated or not treated with steroids, however, the incidence was 93% vs. 35%, respectively.

Although a cataract developed in all patients, a fast developing high-grade cataract was relatively infrequent in patients who were not treated with steroids post transplant.

In **Chapter 3** the data of the 93 patients were reevaluated from a more clinical point of view: what is the severity of the visual impairment as a result of cataract formation after TBI, as experienced by the patient.
Visual problems as a result of the radiation-induced cataracts were for a large part caused by glare. Glare is a visual problem occurring in bright light or focussing a light source in the dark, and is not adequately quantifiable. Testing of the visual acuity could not be used to measure the visual impairment. Therefore, apart from assessing the development of a cataract by making slides, patients were also interviewed for visual complaints as experienced by the patient him- or herself: not present, mild or severe. *Mild* visual complaints were defined as visual impairment, noticed by the patient, but not interfering with normal daily functioning and not a reason for cataract surgery. *Severe* visual complaints were defined as visual impairment interfering with daily functioning or work and as a motivation to undergo cataract surgery. The percentage of the patients with a stable cataract without noticing the existence of a lesion in the eye lens was, at the end of the study, 32%. Mild visual problems were present in 16% and 52% had developed severe visual impairment (SVI) requiring cataract surgery.
The probability of cataract formation at 96 month after TBI, found in the former analysis, was 94%. The probability to develop SVI 96 month after HSCT for patients with a stable cataract was, however, only 44% (for allogeneic patients 33% without and 71% with steroid therapy, and for autologous transplanted patients 37%). The remaining patients all had stable cataract with no or mild visual impairment, without any need for cataract surgery. The SVI-free probability reached a plateau distinct from the probability-curve to develop a cataract.

Single-dose TBI or TBI given in two fractions may result in severe cataract requiring cataract surgery in about 50 % of the patients. In general, shielding during TBI for HSCT is applied to protect critical organs from late effects of the TBI. The disadvantage of shielding is that not only the organs at risk are shielded, but also malignant cells present in the shadow of the blocks. As eyes, just as the CNS, are considered to be “sanctuary sites” were chemotherapy might penetrate less, shielding of the eyes is controversial. In some centers eye shielding is regularly applied, in others not.

Chapter 4 describes a retrospective study on risks and benefits of shielding of the eyes in 188 children with (n = 139) and without (n = 49) eye shielding during TBI in Leiden and Utrecht. The purpose of the study was to determine if there is indeed an increased risk for relapse in the eyes or in the CNS, when shielding is applied during TBI in the anterior field (causing herewith a shadow of reduced dose intensity in the brain). None of the children without shielding had a relapse in the eyes or in the CNS. Of the children with shielding none had a relapse in the eyes and two (1.4%) had a relapse in the CNS, which appears to be within a normal range of CNS relapses after HSCT. Although the numbers were limited, we concluded that shielding of the eyes did not seem to be contraindicated.
To evaluate also the benefits of eye shielding (prevention of cataract induction) we investigated in the evaluable children also the probability of cataract formation and severity of the cataracts, with and without shielding.
Without lens shielding a cataract developed in 90% of the children, of which 38% had severe cataract. Of the children with shielding 31% developed a cataract and only 3% had severe cataract. The probability to stay cataract-free for 5 years after HSCT was 0.77 with and 0.33 without shielding, respectively. The relative risk of developing a cataract without shielding *vs*. with shielding was 3. No effect of steroid treatment could be found, as the number of evaluable patients who needed long-term steroid treatment for GVHD was too small.
Shielding increased the latency time of cataract and decreased the severity.

In **Chapter 5** the assessment of a dose-effect relationship for cataract induction was presented.
To protect radiosensitive organs from late effects of TBI, shielding is applied. Another option to lower the impact of radiation on the eye lenses, is fractionation of the TBI dose (3-6). Fractionation of the total dose as well as reduction of the dose by shielding during single fraction TBI, both result in reduction of the effect of irradiation. The occurrence of a biologic effect of radiation E, like cataract formation, depends on the dose in a linear and quadratic (LQ) fashion (7). The LQ-concept allows converting of each TBI schedule into a single biological effective dose (BED). For cataract induction in the human lens by irradiation, no dose-effect relationship was available. To calculate the cataract incidence for a certain irradiation regimen according to the LQ-concept, are, apart from the radiation parameters, two other parameters are required: the tissue specific parameters α/β and the rate of sublethal damage repair μ (or half-time for repair). For a large number of early and late responding tissues (like lung and kidney tissue) α/β and μ values already had been

reported, however, not for the human lens. To obtain these parameters the data of a large series of patients are required treated with different doses and dose rates. In an earlier analysis by Belkaçemi et al. (8) the data of 2149 patients, treated for acute leukemia were retrospectively evaluated. They were collected by the European Group for Blood and Marrow Transplantation (EBMT). The Acute Leukemia Working Party of the EBMT allowed reusing these collected data. We analyzed 495 of the patients who were treated with single-dose TBI (STBI), with different total doses and dose rates, as part of their conditioning regimen for HSCT. Of these 495 in 175 a cataract was reported. BEDs for different sets of values for α/β and μ were calculated for each patient. With statistical methods (Kaplan-Meier analysis and Cox regression analysis) an α/β of 0.75 Gy and a μ value of 0.65 h^{-1} were found for the eye lens in patients who had no post transplant steroid or heparin treatment (as these both were found to influence cataract formation). Taking into account the found α/β and μ values, and the radiation parameters, we now are able to quantify the incidence of cataract formation. The calculated values also present the possibility to estimate the cataract incidence after reduction of the lens dose by shielding.

In **Chapter 6** we present a study with the aim to find the tolerance dose of kidney tissue when TBI is performed.
Late renal dysfunction after TBI for HSCT was reported to be mainly attributable to radiation nephropathy, characterized by an increase in serum creatinine, proteinuria and hypertension (9,10). Several authors published about late deterioration of renal function as an injury resulting from TBI (11-14). We found eleven reports describing the frequency of renal dysfunction after TBI. We reviewed and compared the results of treatments with various TBI regimens, with the aim to derive a dose-effect relationship for the endpoint late renal dysfunction. For intercomparison of the TBI schedules we applied the LQ concept, to convert each TBI regimen into a BED value for kidney tissue (7) and found the tolerance BED of kidney tissue to be about 16 Gy. Surprisingly, almost all TBI schemes found in the published studies, and all TBI regimens applied in centers for HSCT in The Netherlands, exceed the tolerance BED for kidney tissue. With respect to shielding, it must be noted that this not only results in a lower BED for the shielded organ, but also in a lower BED for the leukemic cells present in the tissues located in the shadow of the blocks. Hyperfractionated TBI seems to be the best option, as by fractionation the BED for the concerned organ lowers, while the BED for leukemic cells can stay relatively high. Only two TBI regimens, among the eleven reports we reviewed, had a BED for kidney tissue of ≤ 16 Gy and can be applied without shielding: 6 x 1.7 Gy or 9 x 1.1 Gy at a dose rate > 5

cGy/min. Their BED for leukemic cells, is, however, low: 11.7 Gy and 11.0 Gy, respectively (15).

In **Chapter 7** we compared the results of HSCT after treatment with various TBI regimens to find dose-effect relationships for the endpoints relapse incidence (RI), relapse-free survival (RFS), non-relapse mortality (NRM) and overall survival (OS).
In the early years of BMT and TBI many different treatment regimens and techniques were used. To find an optimal regimen many authors tried to find a relationship between the total dose of TBI and treatment outcome after HSCT. Some reported a higher OS with increasing TBI dose (16,17), others found opposite results (18,19). In order to find a TBI regimen with optimal results, taking into account a high efficacy and a low toxicity profile, we performed a literature search and compared the results of the different TBI regimens found in the literature. Normalization of the regimens was performed using the LQ-concept (7). We analyzed the results of 3 randomized studies, 4 studies comparing the results of two or three TBI regimens and 9 studies reporting results of one specific TBI regimen. The results of the randomized studies and the studies comparing results of TBI 2 or 3 regimens were pooled and the pooled relative risks (RR) calculated for treatments with a high BED values *vs*. treatments with a low BED. BED-effect relationships were determined. We found that the regimens with higher BED values resulted in a lower RI, an increase in OS and RFS and a lower NRM. The conclusion of this study is that a TBI regimen with a high BED value for leukemic cells, > 15 Gy, is preferred. A relatively high BED for leukemic cells can, however, only be applied taking into account the tolerance BED of normal tissues (20,6). The BED value for lung, during TBI, is in most studies assumed to be 12-14 Gy, the BED for kidney tissue 16-17 Gy (21-23,12) and for severe cataract in the eye lens 40-45 Gy (24). The requirements for an optimal TBI scheme therefore are BED for leukemic cells as large as possible, i.e.15 Gy and larger, and the BEDs for lung, kidney and eye lens tissue not exceeding 15 Gy, 17 Gy and 45 Gy, respectively. As the measure of shielding in highly fractionated regimens is relatively limited highly fractionated schemes must be considered the most effective.

In The Netherlands, various ways are in use to deliver TBI. It seemed therefore useful to have a look at the results in the large Dutch centers for HSCT, and find out if differences in results could be detected, that might have been caused by the different TBI regimens.

To achieve this, in **Chapter 8** the BED-effect relationships of the TBI regimens, used as part of the conditioning for autologous and allogeneic HSCT, for AL and NHL, were studied. The study concerned 1032 patients, treated from 1990-2002. Endpoints were OS, RI, NRM and RFS. A multivariate analysis was performed stratified for disease (AL and NHL) to evaluate the endpoints. For the analysis the BED values of the applied TBI regimens were calculated, and divided into three groups: BED ≤ 14 Gy, 14< BED ≤ 18 Gy and BED >18 Gy. Variables that were analyzed, apart form BED regimen, were patient age, gender and type of transplantation (autologous or allogeneic) as they might have been of influence on the results. Also the BED values for leukemic cells in the shadow of the shielding blocks were calculated, as the measure of organ shielding was one of the major discrepancies between the centers.

Patient age and gender appeared to be only variables significantly influencing OS. The BED group of the TBI did not influence OS or RFS but was only of significant influence on NRM and RI, for both diseases. We found no indication that the effect of the dose group was dependent on the variables age, gender, type of transplantation and type of disease; interaction tests were all with $p > 0.20$.

The results from our earlier study (Chapter 7) could, however, only be partly confirmed: the RI decreased with increasing dose, but there was no effect of the size of the TBI dose on the OS.

We could not find an effect of the measure of shielding of lungs and kidneys on local relapse incidence, nor on OS. Reason was the low number of relapses diagnosed behind the shielding blocks, despite the relatively large volume of hematopoietic tissue and leukemic cells encompassed in the shadow of the blocks. Many more lesions were found but many had to be excluded because of doubt about the diagnosis (GVHD, infection or relapse). Furthermore, once a relapse was suspected after transplantation, the true diagnosis was not always verified when there were no consequences for the treatment.

Relapses in the shadow of the (tiny) eye blocks, were low in number, but were only found when was shielded to a very low dose. They were also mostly solitary. Reason might have been the extremely low BED in the shadow of the eye blocks.

For the analysis of patient age three groups were defined: < 20 years, ≥ 20 and < 40 years, and ≥ 40 years. We found a gradual increase of NRM and a decrease of RFS with advancing age. In multivariate analysis, stratified for disease, the influence of the age groups on OS and RFS was independently significant in favor of younger age for both diseases. Age group was not of influence on RI, but was of a highly significant influence on

NRM. HR for NRM of patients > 40 years was 2.29 (p = 0.001) *vs.* patients < 20 years. We could confirm the effect of TBI dose on RI in children, found by Willemze *et.al.*: the lower RI in older children compared to younger ones, is associated with the higher TBI dose older children receive (25). In our study, in the youngest of the age groups, (adjusted for autologous/allogeneic) the same effect of the TBI dose on RI was found in patients > 10 years compared to patients ≤ 10 years. The effect disappeared when the model was adjusted for TBI dose.

In multivariate analysis, gender was of significant influence on OS, RFS and NRM, for AL and NHL, in favor of male patients. We could not find an explanation for this phenomenon.

OS and RFS of autologous and allogeneic transplanted patients were in multivariate analysis not significantly different. Patients who were transplanted allogeneic had a significantly lower RI but a higher NRM compared to autologous transplanted patients.

With respect to type of disease: patients suffering from NHL had in univariate analysis a significantly better OS and lower RI than patients with AL.

Chapter 9

CONCLUSIONS

- With single-dose or two high fractions TBI, the probability to develop a cataract is 100%.
  Steroid treatment after HSCT significantly shortened the latency-time of cataract-formation and adversely influenced the final severity of cataracts after TBI for HSCT.
- Although with single-dose or two-fractions TBI the probability to develop a cataract was 100%, the probability to develop severe visual impairment as a result of this cataract was only 44%.
  Steroid treatment for GVHD increased the percentage of severe visual impairment, caused by cataract, significantly.
- Eye shielding during TBI in children did not seem to increase the risk for relapse in the eyes or the CNS. Eye shielding prolonged the latency time of cataract and decreased the severity.
- For lens tissue an α/β of 0.75 Gy and a μ of 0.65/h were found, allowing to calculate a dose-effect relationship for cataract formation for specific TBI regimens and specific dose reductions by shielding.
- Nephropathy is a risk when the BED for kidney tissue of a TBI regimen exceeds 16 Gy. As nearly all TBI regimens, found in the literature exceed this dose, kidney shielding is probably unavoidable.
- The requirements for an optimal TBI regimen are: BED-value for leukemic cells as high as possible, i.e.15 Gy or higher, while BEDs for lung, kidney and eye lens tissue should not exceed 15 Gy, 17 Gy and 45 Gy, respectively.
  As the measure of shielding in highly fractionated regimens is relatively limited, highly fractionated schemes must be considered the most effective.
- From the analysis of the data of 1032 patients transplanted in Dutch centers, with single dose TBI or TBI in two fractions, was concluded that
  - The differences in size of the BED values of TBI regimens applied in The Netherlands do not result in differences in OS and RFS after HSCT, for AL as well as NHL; only RI and NRM are influenced significantly. For the higher BED regimens RI is significantly lower but NRM higher.
  - Although it was not feasible to demonstrate a significant inverse influence of shielding blocks on relapse incidence, shielding to very low doses seems not advisable.

- Age is of significant influence on OS, RFS and NRM in favor of younger patients. There is a highly significant influence on NRM, but there is no influence of age on RI, for both diseases.
- Gender significantly influences OS, RFS and NRM in favor of male patients, in AI as well as NHL.
- Type of transplant only significantly influences RI and NRM without influencing OS and RFS, in AL as well as NHL
- NHL patients have a better OS and lower RI after HSCT than patients with AL (univariate analysis).

Chapter 9

GENERAL DISCUSSION

Attempts to perform bone marrow transplantation (BMT) after conditioning with TBI are reported since 1957 (26). From a report in 1977 became clear that some of the patients might be cured (27). In the late 1970^s, BMT finally became an accepted treatment for patients in first remission of leukemia. Nowadays in Europe, hematological stem cell transplantation (HSCT) is applied in an early stage of several diseases with greatly improved results. In 2005, 24168 first HSCTs were reported, 8890 allogeneic (37%), 15 278 autologous (63%) and 3773 additional re- or multiple transplants. Main indications were leukemia's, lymphomas, solid tumors and non-malignant disorders (28). The number of allogeneic transplants is still increasing, while autologous transplants are stable. There is an increasing use of unrelated donors and lately there are also major changes in the conditioning regimens.

Total body irradiation always was an important but toxic element in the preparation of patients for bone marrow transplantation. It can be delivered in many different ways and analyzed for many different endpoints such as acute and late toxicity of radiosensitive organs and overall survival (OS), disease-free survival (DFS), transplant-related toxicity (TRM) and relapse incidence (RI).

When TBI started in The Netherlands in the 1970^s (Leiden), still relatively little was known about the toxicity of the combination BMT and TBI and the additive effect of drugs and radiation. It was obvious that the TBI dose given before BMT was too high for the lungs because of the danger of radiation pneumonitis. As a consequence lungs were shielded to a dose regarded as safe. Yet still pneumonitis occurs usually within the first four month and can be lethal (29). A large proportion of cases is caused by CMV infection, a small proportion by other viruses. In most of the remainder the process is idiopathic, probably representing a combination of adverse effects of GVHD, drug toxicity and damage by irradiation.

When TBI for BMT started in Utrecht in 1981, we decided to prospectively investigate the eye lenses of the patients for cataract formation

Cataract

TBI as a part of the conditioning regimen was by several authors described as a cause of cataract formation (30,31,3). Steroid treatment is also known as a cause of cataract formation and is described to have an additive and synergistic effect on cataractogenesis

after TBI (32,33,8). Usually cataract formation after HSCT was reported retrospectively from patients having complaints, or needing cataract surgery. Few data were published prospectively.

In our study (Chapter 2) the probability of cataract formation after TBI, applied single-dose or in two high fractions, was found to be 100%. The cataracts appeared to stabilize after ≤ 30 month in varying grades of severity. The grade of severity a cataract attained varied, depending on steroid treatment yes or no. The visual impairment as a result of a cataract (defined as: not present, mild or severe - needing surgery) varied largely (Chapter 3). Of the patients with steroid treatment 86.6% developed cataract with severe visual impairment (SVI) *vs*. 39% without treatment with steroids (autologous and allogeneic transplanted). Of all patients with a cataract, 48% had not any or only mild visual impairment.

Cataract is not a life-threatening lesion and cataract surgery nowadays results in a very high success percentage (34,35). It is, however, one of the problems that happen to a patient, often just when the most stressful life-threatening period is over. It would be better if cataract surgery could be prevented. In young patients an artificial lens leads to early loss of accommodation, in children cataract surgery often has to be postponed until they are full-grown. In the Netherlands it therefore is common use to shield the eyes of children to a dose considered to be less cataractogenic and, because of the apparent success of this policy, in a number of centers also the eyes of adults are shielded. However, shielding of the eyes results also in a reduction of the dose to the leukemic cells that are present in the shadow of the shielding block and thus contains a risk for relapse. Eyes are considered to be an extramedullary sanctuary, just as the CNS and eye shielding, therefore, is controversial. An increased incidence of relapse in the eyes as a result of shielding could not be confirmed in the study we conducted in 188 children, with or without lens shielding during TBI (Chapter 4). In our last study, however, including patients with eye shielding to very low doses (BED for leukemic cells < 6 Gy) we found 3 solitary relapses in the shadow of the tiny blocks in the eye region (Ø 3 cm), while we did not find any in patients with higher doses on the eye region. The finding was not statistical significant, but indicates that we better should not shield the eyes to low doses.

Another option to avoid severe cataract, besides shielding, is lowering the impact of the irradiation by fractionation of the TBI dose (3-6). Fractionation of the total dose reduces the detrimental effect of irradiation on healthy tissue. To predict the cataract incidence of a TBI scheme, fractionated and single dose, it should be possible to calculate the BED for lens

tissue of the concerning TBI scheme, using the BED-LQ concept. For this calculation, however, a tissue specific parameter α/β and the rate of sublethal damage repair μ (or half time of repair) has to be available (7). For lens tissue these parameters were unknown yet. With the use of a large number of data placed at our disposal by the Acute Leukemia Working Party of the EORTC, we could, however, derive an α/β (0.75 Gy) and a μ value (0.65 h^{-1}) for eye lens tissue (Chapter 5). For each TBI regimen, fractionated or single-dose, the cataract incidence can therefore now be quantified. Also the cataract incidence when the lens dose is reduced by shielding can in this way be estimated. It is therefore possible to determine a TBI regimen with minimal cataract induction.
For a TBI regimen with an optimal leukemic cell kill a TBI regimen with a BED for leukemic cells (BED_{leuk}) of > 15 Gy is required (15), the BED for eye lens (BED_{eye}) with minimal induction of severe cataract is 40-45 Gy (24).

Examples of BED regimens and accessory BEDs for eye lens (BED_{eye}), dose rate e.g. 5 Gy/h:

- 2 x 6 Gy: BED_{leuk} = 16.4 Gy, BED_{eye} = 87.2 Gy.
  To reduce the BED_{eye} to minimize the risk for severe cataract the lens has to be shielded e.g. to 2 x 4 Gy (BED_{eye} = 41.4 Gy).
- 6 x 2 Gy: BED_{leuk} = 14.0 Gy, BED_{eye} = 41.4 Gy.
- 6 x 2.2 Gy: BED_{leuk} = 15.6 Gy, BED_{eye} = 48.5 Gy.
- 9 x 1.6 Gy: BED_{leuk} = 16.4 Gy, BED_{eye} = 43.1 Gy.

Late effects on kidney tissue

The initial opinion was that the TBI dose did not exceed the tolerance dose of kidney tissue and therefore could be given safely (36,37). Soon it became evident that the tolerance dose for the combination of TBI, chemotherapy and stem cell transplantation for several tissues, e.g. lung tissue, was lower than expected, especially when allogeneic transplantation was performed. Because the tolerance dose for kidney, with irradiation alone, approximated the TBI dose, some felt it would be safer to apply kidney shielding. Indeed, from about the late 1980s several authors reported late renal dysfunction, apparently due to radiation damage (11-14), and also reduction of late renal failure when shielding was applied (12,21,23). A disadvantage of shielding, which was mentioned before, is that in the shadow of the blocks also a reduction in dose to circulating leukemic cells is created. Just as for the prevention of cataract, fractionation of the TBI dose was reported to be successful in increasing the tolerance of kidney tissue (37-41). An optimal TBI scheme was, however, not established yet. The regimens mentioned in most studies varied, inclusive the dose in the shadow of the shielding blocks. In order to find the tolerance dose for kidney tissue, and to find an optimal TBI treatment regimen, we reviewed the literature and compared the TBI regimens with respect to late renal dysfunction (Chapter 6). Normalization of the TBI doses was performed using the LQ-BED concept. From the dose-effect relationship that was obtained it was concluded that the tolerance dose for kidney tissue (BED_{kidney}) was 16-17 Gy. An unexpected finding was that in almost all regimens the BED for kidney tissue was too high. Even in centers using kidney shielding the BED value for kidney tissue could still be too high. For instance, Kersting *et al.* (42) reported that 23% of the patients with kidney shielding to 2 x 5 Gy, and dose rate 9 Gy/h, (BED for kidney = 28.1 Gy) had chronic kidney disease after HSCT, with a 10-years probability of 27%. As mentioned by Kal *et al.* (43), kidney shielding in the concerned patients should have been lowered to 2 x 3.6 Gy (BED for kidney = 16.6 Gy) to prevent late renal dysfunction.

Anyway, shielding has to be applied to prevent renal damage. TBI schemes that do not need kidney shielding (e.g. 6 x 1.7 Gy or 9 x 1.1 Gy, dose rate > 0.05 Gy/min.), have a BED of <15 Gy for leukemic cells that probably is too low. Therefore, kidney shielding seems unavoidable. The dose reduction that has to be applied for shielding the kidneys, when fractionated TBI is applied, is relatively small. Highly fractionated schemes, therefore, must be considered the most effective.

Examples of TBI regimens, accessory BEDs for kidney tissue (BED_{kidney}) at a dose rate of 5 Gy/h and BED_{kidney} after shielding to about 17 Gy:

- 2 x 6 Gy: BED_{kidney} = 36.2 Gy, shielded to 2 x 3.8 Gy, BED_{kidney} = 17.3 Gy
- 6 x 2 Gy: BED_{kidney} = 20.0 Gy, shielded to 6 x 1.75 Gy, BED_{kidney} = 17.4 Gy
- 6 x 2.2 Gy: BED_{kidney} = 24.1Gy, shielded to 6 x 1.75 Gy, BED_{kidney} = 17.4 Gy
- 9 x 1.6 Gy: BED_{kidney} = 23.2 Gy, shielded to 9 x 1.3 Gy, BED_{kidney} = 17.4 Gy

Dose-effect relationships of TBI for leukemic cells and transplant-related mortality

Many authors reported about a relationship between the total TBI dose and the treatment outcome after HSCT (16-19,25,44).
We conducted a study reviewing the results of literature-derived TBI regimens in order to find a TBI regimen with optimal results (Chapter 7). For normalization we used the LQ concept, calculating the BED values for leukemic cells of every TBI regimen. Studies with patients treated for AL with allogeneic HSCT were selected and the data were pooled. Regimens with high BED values appeared to cause less leukemia relapses and had a higher RFS and OS. Regimens with high BED values, therefore, are of importance, especially for the prevention of leukemic relapse and apparently without increase of the NRM. A relatively high BED for leukemic cells can, however, only be administered when the tolerance BED of normal tissues is brought into account. For applying these relatively high BED values, shielding of lungs and kidneys is unavoidable, also when TBI is fractionated. In most studies, the lung dose is restricted to a dose corresponding with a BED value for lung tissue of <14 Gy. In a number of publications it was recommended to limit the BED value for kidney tissue to 16-17 Gy (21-23,12). The incidence of severe visual impairment by cataract formation is negligible for BED values of lens tissue of < 45 Gy (24). The requirements for an optimal TBI scheme therefore are: BED for leukemic cells as large as possible, i.e. BEDs of 15 Gy and larger, and BED for lungs, kidneys and eyes not exceeding 15, 17 and 45 Gy, respectively. This means that TBI regimens applied

as a single dose, and nearly all fractionated TBI regimens currently used, should all be applied with shielding of lungs and kidneys. Highly fractionated schemes are the most effective for this purpose, as the measure of shielding sensitive organs can be relatively limited compared to single dose and low fraction number regimens.

In The Netherlands, as everywhere, the methods of TBI vary among institutions. This probably is inherent to the complicated procedure, local conditions and patient load. Organ shielding is one of the major variables. It seemed, therefore, meaningful to investigate and compare the results of the conditioning regimens of patients transplanted in The Netherlands, who had high dose TBI as part of their conditioning regimen (Chapter 8).
Common practice in The Netherlands is to apply TBI in one or two large fractions with a relatively high dose rate. Evaluated were the data of 1032 patients with AL and NHL, transplanted autologous or allogeneic, from 1990 until 2002. The BED values of the TBI regimens were normalized and, as also the dose reductions for sensitive organs varied, the dose in the shadow of the shielding blocks was normalized as well. The BED value of the TBI was investigated as a variable in multivariate analysis, stratified for disease, AL and NHL. Variables that might have influenced the results and therefore were tested as well were, age, gender and type of transplantation.
Reviewing the results of the various Dutch TBI regimens, we could only partly confirm the results from our earlier investigation in Chapter 7: that higher BED values of the TBI regimen are resulting in a lower RI and a higher RFS and OS. We found in the Dutch patients indeed a relatively low RI in the schemes with the highest BED compared to the lower BED regimens; the low RI in these high BED patients was, however, accompanied with a relative high NRM, offsetting the effect of the low RI. No effect of the BED value on OS and RFS could be found. The effect of the dose group was not dependent on the variables age, gender and transplantation type as all interaction tests performed were within $p > 0.20$).
A difference between the study using the literature derived data (Chapter 7) and the study evaluating the data of the Dutch patients was, that patients in The Netherlands only had TBI in one or two large fractions at a relatively high dose rate, whereas the patients from the selected studies, in the majority of cases received a hyperfractionated TBI regimen. When single fraction was applied, they received also distinctly lower dose rates. BEDs for leukemic cells might be equally high in single-dose high dose rate TBI and in fractionated TBI, the toxicity for normal tissues, however, differs. This might have played a role.

The effect of the dose reduction by the shielding blocks on RI was complicated. The number of extramedullary and extranodal relapses, diagnosed in the shadow of the blocks, was low. Many lesions were observed, but it proved difficult to distinguish lesions caused by relapse from effects of GVHD or infection. Also came forward that once was obvious that a relapse had occurred, for instance from the appearance of leukemic cells in the peripheral blood, often no further diagnostic search for site of relapse was done. Reason was the fact that there were no consequences for the treatment; patients could often only be treated palliative. Also, when relapse was expected but treatment not possible, e.g. because of severe infections or GVHD, site of relapse was frequently not verified. The number of local relapses was, therefore, probably underestimated and the exact site of relapse often unknown.
An effect of the size of the BED behind lung or kidney blocks on the local relapse incidence could therefore not be found. Another problem with respect to the effect of dose reduction by lung blocks was that, although the BED of the TBI regimens differed largely, the BED in the shadow of the lung blocks did not show much variation. This made it difficult to compare OS and DFS of the TBI according to effect of the dose reduction resulting from lung shielding.
Lesions in the eye region that with certainty could be diagnosed as relapse, were low in number (n = 4), but only located in the shadow of a shielding block and correlated with an extremely low BED for leukemic cells. As also three of the four were solitary, there seemed to be an argument not to apply too low BED values in the shadow of the shielding blocks, although there could be no statistical significance. Numbers were too small.

Age and gender were the only variables that independently influenced OS, RFS and NRM in multivariate analysis significantly, for both diseases. We divided the patients according to age into three groups: < 20 years, ≥ 20 and < 40 years, and ≥ 40 years. It appeared that age had a highly significant influence on NRM in favor of younger patients. There seemed, however, to be hardly any influence on RI. Several authors discussed the influence of age: there is general agreement about the superior tolerance of children for transplant-related toxicity and about the bad tolerance of patients over sixty years of age (45). There is no study that found an influence of the risk of TRM and DFS post transplant, with gradually increasing age (45). The effect on TRM, RI and DFS for all ages was in our study, however, pictured quite nicely. The superiority of the results with respect to TRM for younger patients is a fact that has to be kept in mind in connection with reduced intensity conditioning (RIC). The success of RIC before HSCT is based on its low toxicity. The

approach is, however, known to be accompanied by a high RI (46-49), probably owing to the lack of the antileukemic potential. As young patients, evidently, already have a low NRM, a RIC regimen seems not of any profit for them. In young patients, the effect of a high-dose conditioning on leukemic cell kill is not offset by the toxicity of the treatment as it probably is in older patients. On the contrary, Willemze et al. (25) found a lower RI in older children compared to younger ones that was associated with the higher TBI dose older children received. We could, in our study, confirm this effect. In the youngest age group, patients > 10 years had a 2-fold reduced RI compared to patients ≤ 10 years when was stratified by disease and adjusted for autologous/allogeneic transplantation. This effect almost disappeared when was adjusted for TBI dose.
The effect of gender found for both diseases in favor of male patients is remarkable but unexplained. There might be a link with a higher susceptibility after HSCT for viral infections of females, reported by Gratwohl (50). It would be in accordance with the higher NRM we found for females, but has to be investigated. The fact that the variable 'type of transplantation' had no significant influence on both diseases, AL and NHL, is also remarkable, but reported before. Also with respect to type of transplantation the benefits of a treatment (high-dose conditioning and graft versus tumor (GVT) effect) are not necessarily additive but can offset one another by the toxicity of one of the two treatments or both.

The strange fact now appears, that all three approaches: autologous and allogeneic HSCT after high-dose conditioning and allogeneic HSCT after RIC, all seem to have about the same outcome (50), while RIC is increasingly popular because of its low toxicity. But, as already mentioned, prudence is called for. When patients of younger age groups are considered, the situation changes, as their NRM is already considerably lower. Young patients, therefore, should not be denied the antileukemic effect of the high-dose conditioning regimen. They probably can benefit from the GVT effect of allogeneic transplantation and they are, probably, also the patients that benefit from the antileukemic effect of a relatively high BED value of the TBI.
Recently, significantly better results in the management of NRM after high dose conditioning over the years were reported. The effect was found to be primarily due to better management and prevention of infections (50,52,53), and most pronounced in patients in first CR (of AL) and therefore, not heavily pretreated (53).
From our study is clear that younger patients have a lower NRM but no lower RI. Other authors report the relapse incidence as a major threat to a successful outcome (54,55).

Most probably, high dose conditioning is the only option to conquer relapse.
The best policy, therefore, seems to be: allogeneic transplantation after high-dose conditioning, using a highly fractionated TBI regimen. In this way TBI with a high BED can be delivered, while low dose regions in the shadow of the necessary blocks will be minimized and the late toxicity of the high-dose TBI will be as low as possible, also for other non-blocked tissues like for instance the thyroid gland. High-dose conditioning should, however, probably be applied in accurately defined groups of patients. Young patients in first complete remission are treated poorly when submitted to a RIC regimen, older or heavily pretreated patients, however, may benefit from RIC. A careful selection is warranted. Trials have to be conducted to be able to select patients groups properly for the treatment they should get and to define cut-off points for high and low-dose conditioning treatments.

Allogeneic HSCT has been able to cure thousands of patients with otherwise lethal disease. With increased numbers of patients surviving on the long term, late effects after HSCT have become of major importance. When in the future high dose conditioning indeed is reserved for relatively young patients in good condition and early remission, OS after high dose conditioning will probably rise even further. The aim in this century should therefore not only be to cure patient's disease, but also to minimize the incidence of post transplant complications and ensure the best possible quality of life.
The main risk factors for post transplant complications are way of pretransplant conditioning and chronic graft versus host disease including its treatment. The role of radiotherapists will be to optimize TBI by applying the treatment that is likely to cause the least late effects, without costs for the antileukemic potential of the TBI. Multifractionated.

Suggestion for suitable schemes (dose rate of e.g. 5 Gy/h) could be:

- 6 x 2.2 Gy: BED_{leuk} = 15.6 Gy
  - BED_{eye} = 48.5 Gy
  - BED_{kidney} = 24.1 Gy (shielded to 6 x 1.5 Gy, BED_{kidney} = 17.4 Gy)
  - BED_{lung} = 21.0 Gy (shielded to 6 x 1.75 Gy, BED_{lung} = 15.4 Gy)
- 9 x 1.6 Gy: BED_{leuk} = 16.4 Gy
  - BED_{eye} = 43.1 Gy
  - BED_{kidney} = 23.2 (shielded to 9 x 1.3 Gy, BED_{kidney} = 17.4 Gy)
  - BED_{lung} = 20.7 (shielded to 9 x 1.3 Gy, BED_{lung} = 15.8 Gy)

TBI with some shielding of lungs and kidneys probably meets this purpose. In two centers for stem cell transplantation in the Netherlands multifractionated TBI, as part of myeloablative conditioning for all patients, children and adults, was recently started

A consideration could be not to shield during every fraction, but e.g. only during two or three fractions, once daily.

To compare (for a dose rate of 5 Gy/h):

- 2 x 6 Gy: BED_{leuk} = 16.4
  - BED_{eye} = 87.2 (shielded to 2 x 4.0, BED_{eye} = 41.4)
  - BED_{kidney} = 36.2 (shielded to 2 x 3.8, BED_{kidney} = 17.3)
  - BED_{lung} = 29.3 (shielded to 2 x 3.9, BED_{lung} = 15.1)

Chapter 9

RECOMMENDATIONS BASED ON OUR FINDINGS
(and the literature)

- Multifractionating of TBI appears to be a good policy, as by multifractionation the BED of sensitive tissues lowers, as compared to the BED using single dose or hypofractionated TBI. The measure of shielding can, therefore, be less or even absent (eyes), minimizing the inhomogeneity of the doses in the target volume caused by the blocks.

- The BED of the kidneys during TBI should be reduced to a BED value for kidney tissue of 16-17 Gy, to prevent late renal dysfunction.

- Multifractionated TBI should have a BED value for leukemic cells of, preferably, 15 Gy or more.

- To compare the results of HSCT properly, agreements should be made to uniformize the conditioning regimens (TBI regimens and techniques, dose reductions by shielding, applied chemotherapy regimens), manipulation of the grafts and other pre- and posttransplant measures, as much as possible.

- In papers reporting about the results of HSCT after TBI, all treatment parameters of the TBI should always be mentioned: total dose, number of fractions, dose rate and, if applicable, measure of shielding.

- Patients who had HSCT with or without conditioning with TBI, should be followed at fixed times throughout their entire lives, to find late effects (e.g. hypertension) in time for treatment, and to adjust conditioning treatments in the future, if possible.

RECOMMENDATIONS FOR FUTURE RESEARCH

- Prospective trials have to be conducted to define patients groups that benefit from high-dose conditioning and patients who will have more benefit from reduced intensity conditioning.

- Prospective trials are needed to find a high-dosed TBI scheme with optimal results with respect to minimal late effects, maximal leukemic cell kill and a practical clinical setting, taking into account the BED for leukemic cells as well as organs at risk.

- The significant effect of gender on overall survival, disease-free survival and non-relapse mortality in favor of male patients is remarkably. As women generally (when no high dose chemo/radiotherapy causing immune deficiency and sex hormone deficiency is concerned) have better prognostic features than males, research with respect to this subject seems interesting.

Chapter 9

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Chapter 9

NEDERLANDSE SAMENVATTING

Samenvatting

Hoofdstuk 1 bevat een introductie tot de hematologische stamcel transplantatie (HSCT) en totale lichaamsbestraling (TBI): complicaties, late effecten en uitkomst van de behandeling en vat tevens het doel van proefschrift samen, bestaande uit 6 hoofdvragen:

1. Wat zijn de karakteristieken van cataractontwikkeling na TBI, indien gegeven in één of twee fracties, als onderdeel van de voorbereiding voor HSCT en wat zijn de consequenties van een bestralingscataract met betrekking tot de visusbeperking.
2. Is er een risico verbonden aan het preventief afdekken van de ogen tijdens de TBI.
3. Is het mogelijk om de incidentie van cataractvorming voor een bepaald TBI schema te voorspellen.
4. Wat is de tolerantiedosis voor nierweefsel als TBI voor HSCT wordt toegepast.
5. Wat zijn de vereisten voor een optimaal TBI regime met betrekking tot een maximale destructie van leukemische cellen, een optimale overall survival (OS) en minimale late schade aan stralingsgevoelige organen als longen, nieren en ooglenzen.
6. Wat zijn de resultaten van HSCT/TBI in Nederland.

Hoofdstuk 2 beschrijft de resultaten van een prospectieve studie over cataractvorming bij 93 patiënten, die werden behandeld met TBI, toegediend in één of twee fracties, als onderdeel van de conditionering voor autologe (n = 42) of allogene (n = 51) HSCT op de afdeling Radiotherapie van het Universitair Medisch Centrum in Utrecht. De patiënten werden behandeld vanaf 1981, toen BMT startte in Utrecht, tot 1995. In de eerste periode werden de cataracten vastgesteld met behulp van dia's van de ooglenzen, die regelmatig op de afdeling Radiotherapie werden gemaakt met een funduscamcera. Na 1989 maakte de fotograaf van de afdeling Oogheelkunde de dia's. Om de cataractvorming vast te stellen en voor follow-up van de cataracten, werden de dia's gemaakt bij ieder bezoek, tenminste jaarlijks, of afwijkingen werden gezien of niet. Om de cataracten te graderen gebruikten wij het classificatiesysteem van Schipper (1,2). Het systeem was gebaseerd op foto's van het posterior-subcapsulair bestralingscataract van de menselijke lens en bestond uit 8 graden van ernst (O/ I - I/V).

De kans om een cataract te ontwikkelen bleek 100% te zijn. Steroïden, toegediend voor graft versus host disease (GVHD), beïnvloedden de latentie-tijd en de uiteindelijke ernst van een cataract. Bij autoloog getransplanteerde patiënten ontwikkelde een cataract zich na een mediane periode van 58 maanden. Bij allogeen getransplanteerde patiënten ontwikkelde het zich mediaan 46 maanden na de HSCT zonder steroïdtherapie en na

mediaan 33 maanden met toediening van steroïden. Gevonden werd dat een cataract zich ≤ 30 maanden, nadat het voor de eerste maal was vastgesteld, stabiliseerde. Bij autoloog getransplanteerde patiënten was de incidentie van stabiel hooggradig cataract 48%. Bij allogeen getransplanteerde patiënten, wel of niet behandeld met steroïden, was de incidentie van hooggradig cataract, respectievelijk, 93% en 35%.
Hoewel een cataract zich ontwikkelde bij alle patiënten, kwam een zich snel ontwikkelend hooggradig cataract dus minder frekwent voor bij patiënten die na de transplantatie niet behandeld waren met steroïden.

In **Hoofdstuk 3** werden de gegevens van de 93 patiënten opnieuw geëvalueerd vanuit een meer klinisch standpunt: wat is de ernst van de visusbeperking ten gevolge van cataractvorming na TBI, zoals de patiënt die ervaart.
De visusproblemen als gevolg van een door de bestraling geïnduceerd cataract worden voor een groot deel veroorzaakt door “glare”. Glare of “schittering” is een visusprobleem dat optreedt bij helder licht, of bij het instellen van de ogen op een felle lichtbron in het donker, en is niet goed kwantificeerbaar. Het testen van de gezichtsscherpte kan daarom niet worden gebruikt om de mate van visusbeperking te meten. Behalve het vaststellen van een cataract met behulp van dia’s werden de patiënten daarom ook gevraagd naar visusklachten, zoals zij die zelf beoordeelden: niet aanwezig, mild of ernstig. *Milde* visusklachten werden gedefinieerd als: visusbeperking, door de patiënt bemerkt, maar niet hinderlijk bij normaal dagelijks functioneren en geen reden om cataractchirurgie te ondergaan. *Ernstige* visusklachten werden gedefinieerd als visusbeperking, hinderlijk bij dagelijkse functioneren of werk en voor de patiënt wel een motivatie om cataractchirurgie te ondergaan. Het percentage patiënten met stabiel cataract dat het bestaan van een afwijking in de ooglens in het geheel niet opmerkte, was 32% aan het einde van de studie. Milde visusklachten waren aanwezig bij 16% en 52% had ernstige visusklachten die cataractchirurgie vereisten.
De kans op cataractvorming 96 maanden na TBI, zoals gevonden in de vorige analyse, was 100%. De kans op de ontwikkeling van ernstige visusklachten (SVI) voor patiënten met een stabiel cataract was echter slechts 44% (bij allogene patiënten 71% met en 33% zonder steroïdtherapie; bij autologe patiënten 37%). De overblijvende patiënten hadden alle een stabiel cataract zonder of met een milde visusbeperking zonder behoefte aan cataractchirurgie. De waarschijnlijkheidscurve om SVI-vrij te blijven bereikte een plateau verschillend van de waarschijnlijkheidscurve om een cataract te ontwikkelen.

Samenvatting

TBI gegeven in één of twee fracties met een hoog dosistempo kan resulteren in een ernstig cataract dat cataractchirurgie vereist bij ca 45% van de patienten. In het algemeen wordt afdekking tijdens TBI gebruikt om gevoelige organen te beschermen tegen late schade als gevolg van de TBI. Het nadeel van afdekken is echter, dat niet alleen de betreffende organen, maar ook maligne cellen, aanwezig in de schaduw van de blokken, worden afgeschermd. Daar ogen, evenals het centraal zenuwstelsel (CZS), worden beschouwd als zogenaamde sanctuary sites, waar chemotherapie minder goed doordringt, is afdekken van de ogen controversieel. In sommige centra worden ogen routinematig afgedekt, in andere niet.

Hoofdstuk 4 beschrijft in een retrospectieve studie de risico's en voordelen van afdekken van de ogen bij 188 kinderen met (n = 139) en zonder (n = 49) oogblokken tijdens TBI. Het doel van de studie was vast te stellen of er een toegenomen risico van recidief in de ogen en /of het CZS optreedt, als tijdens TBI oogblokken worden toegepast in het voorveld (die ook een schaduw van verminderde dosisintensiteit in de hersenen veroorzaken). Geen van de kinderen zonder oogblokken had een recidief in de ogen of in het CZS. Van de kinderen met oogblokken had geen enkele een recidief in de ogen, twee (1.4%) hadden een recidief in het CZS. Dit laatste blijkt binnen de normale spreiding voor CZS recidieven na BMT te vallen. Op grond van dit onderzoek lijkt afdekken van de ogen daarom niet gecontraindiceerd te zijn, hoewel de aantallen beperkt waren.
Teneinde ook de voordelen van oogafscherming te evalueren (voorkomen van cataractvorming), onderzochten wij bij de kinderen die evaluabel waren, met en zonder oogblokken, ook de kans op cataractvorming en de ernst van de cataracten.
Zonder oogafscherming ontwikkelde zich een cataract bij 90% van de kinderen, van wie 38% een ernstig cataract had. Van de kinderen met lensafscherming ontwikkelde 31% een cataract; slechts 3% had een ernstig cataract. De kans om gedurende 5 jaar na HSCT cataract-vrij te blijven was 0.77 met en 0.33 zonder oogblokken. Het relatieve risico voor cataractvorming zonder versus met afscherming was 3. Een effect van behandeling met corticosteroïden kon niet worden gevonden, daar het aantal evaluabele patiënten, die langdurige behandeling met corticosteroïden nodig hadden, te klein was.
Afscherming vergrootte de latentietijd van het cataract en verminderde de ernst.

In **Hoofdstuk 5** wordt een dosis-effect relatie voor cataractinductie gepresenteerd. Om stralingsgevoelige organen te beschermen tegen late schade als gevolg van de TBI wordt afscherming toegepast. Een andere optie om de impact van straling op de ooglenzen te

beperken is fractionering van de TBI dosis (3-6). Fractionering van de totaaldosis zowel als reductie van de dosis door afscherming tijdens TBI toegediend in één fractie, resulteren beide in reductie van het effect van de bestraling. Het optreden van een biologisch effect van straling E, zoals cataract, hangt af van de dosis, op een lineair quadratische (LQ) wijze (7). Het LQ-concept staat omrekening (of normaliseren) van ieder TBI schema in één biologisch effectieve dosis (BED) toe. Voor inductie van cataract ten gevolge van straling in de menselijke lens was nog geen dosis-effect relatie beschikbaar. Om de incidentie van cataract voor een bepaald bestralingschema volgens het LQ-model uit te rekenen, zijn, behalve de bestralingsgegevens namelijk twee andere parameters vereist: de weefselspecifieke parameters α/β en de snelheid van herstel van subletale schade μ (of half-waarde tijd voor herstel). Voor een groot aantal vroeg en laat responderende weefsels (zoals long en nierweefsel) zijn de α/β- en μ-waarden al bekend, voor de menselijke lens was dat echter nog niet het geval. Om deze parameters te verkrijgen zijn de gegevens nodig van een grote serie patiënten, behandeld met verschillende totaaldoses en dosistempi. In een eerdere analyse door Belkaçemi et al. (8) werden de gegevens van 2149 patiënten, behandeld voor acute leukemie, retrospectief geëvalueerd. Zij waren verzameld door de European Group for Blood and Marrow Transplantation (EBMT). De Acute Leukemia Working Party van de EBMT stond ons toe deze verzamelde data te hergebruiken. Wij analyseerden 495 van de patiënten die waren behandeld met TBI in één fractie (STBI), met verschillende totaaldoses en dosistempi, als onderdeel van hun conditionering voor HSCT. Van deze 495 patiënten werd bij 175 een cataract gediagnostiseerd. BEDs voor verschillende sets van waarden van α/β en μ werden berekend voor iedere patiënt. Met statistische methoden (Kaplan-Meier analyse en Cox regressie analyse) werden een α/β van 0.75 Gy en een μ van 0.65/h^{-1} voor de ooglens gevonden, bij patiënten die na de transplantatie niet behandeld waren met steroïden of heparine (dit, omdat door Belkaçemi et al. gevonden was dat steroïden en heparine de cataractvorming beïnvloedden). Met behulp van de gevonden α/β- en μ-waarden en de bestralingsparameters, is het nu mogelijk ook de incidentie van cataract te berekenen. De berekende waarden geven ook de mogelijkheid de incidentie van cataract na reductie van de lensdosis, b.v. door afscherming, te bepalen.

In **Hoofdstuk 6** presenteren wij een studie met als doel de tolerantiedosis van nierweefsel voor TBI i.v.m. HSCT te bepalen.
Radiatie nefropathie wordt gekarakteriseerd door een toename in serum creatinine proteiurie en hypertensie (9,10). Door verschillende auteurs wordt TBI als onderdeel van de

conditionering voor HSCT vermeld als belangrijkste risicofactor voor laat optredende nierfunctiestoornissen (11-14). We vonden 11 publicaties die de frekwentie van late nierfunctiestoornissen na TBI beschreven en bekeken en vergeleken de resultaten van behandeling met verschillende TBI schema's. Getracht werd een dosis-effect relatie te vinden voor het eindpunt late nierfunctie afwijkingen na TBI. Voor onderlinge vergelijking van de TBI schema's pasten wij het LQ-concept toe en normaliseerden elk TBI regime tot een BED voor nierweefsel (7). Gevonden werd dat de tolerantie-BED voor nierweefsel ca. 16 Gy was. Verrassend was dat bijna alle in de literatuur gepubliceerde TBI schema's en alle TBI regimes toegepast in centra voor HSCT in Nederland de tolerantie-BED voor nierweefsel tijdens TBI overschrijden. Afdekken van de nieren is dus noodzakelijk om late nierschade te voorkomen. Opgemerkt moet worden dat afscherming niet alleen resulteert in een lagere BED voor het afgedekte orgaan, maar ook in een lagere BED voor leukemische cellen en beenmerg, aanwezig in de weefsels in de schaduw van de blokken. TBI gegeven in multipele fracties lijkt de beste optie, daar door fractionering de BED van de TBI voor het betrokken orgaan lager wordt, terwijl de BED voor leukemische cellen relatief hoog kan blijven. Slechts twee TBI regimes van de 11 die wij opnieuw bekeken, hadden een BED voor nierweefsel van ≤ 16 Gy en kunnen dus worden toegepast zonder afdekking: 6 x 1.7 Gy en 9 1.1 Gy, dosistempo >5 cGy/min. Hun BED voor leukemische cellen is echter relatief laag: 11.7 en 11.0 Gy respectievelijk (15).

In **Hoofdstuk 7** vergeleken wij de resultaten van HSCT na behandeling met verschillende TBI regimes, teneinde dosis-effect relaties te vinden voor de eindpunten: overleving in het algemeen (OS), incidentie van het recidief (RI), recidief-vrije overleving (RFS) en mortaliteit ten gevolge van andere oorzaken dan recidief (NRM).
In de vroege jaren van de toepassing van HSCT en TBI werden veel verschillende behandelings-schema's gebruikt. Om een optimaal schema te vinden probeerden veel auteurs een relatie te vinden tussen de totale TBI-dosis en en de uitkomst van de behandeling na HSCT. Sommigen rapporteerden een hogere OS met toenemende TBI-dosis (16,17), anderen vonden tegenovergestelde resultaten (18,19). Teneinde een TBI regime te vinden met optimale resultaten, dat een hoge effectiviteit paart aan een lage toxiciteit, deden wij een literatuur onderzoek en vergeleken de resultaten van de verschillende TBI regimes die wij vonden. Normalisatie van de regimes werd uitgevoerd met behulp van het LQ-concept (7). We analyseerden de resultaten van drie gerandomiseerde studies, 4 studies die de resultaten vergeleken van twee of drie TBI regimes en 9 studies die de resultaten rapporteerden van één specifiek TBI regime. De

resultaten van de gerandomiseerde studies en de studies die de resultaten van 2 of 3 regimes vergeleken werden gepoold. De gepoolde relatieve risico's (RR) werden berekend voor behandelingen met hoge BED-waarden versus behandelingen met een lage BED en de BED-effect relaties werden bepaald. Wij vonden dat regimes met hoge BED-waarden resulteerden in een lagere RI, een toename in OS en RFS en een lagere NRM. De conclusie van deze studie is daarom dat een TBI-schema met een hoge BED-waarde voor leukemische cellen de voorkeur heeft. Een relatief hoge BED voor leukemische cellen kan echter alleen worden toegepast als rekening wordt gehouden met de tolerantie BED voor normale weefsels (20,6). In de meeste studies wordt bij TBI, voor de long een BED-waarde aan gehouden van 12 – 14 Gy, voor nierweefsel 16 – 17 Gy (21-23) en voor de ooglenzen (d.w.z. cataract met ernstige visusklachten) 40 – 45 Gy (24). De vereisten voor een optimaal TBI schema zijn daarom: BED voor leukemische cellen zo hoog mogelijk b.v. 15 Gy en hoger, en de BEDs voor long-, nier- en ooglensweefsel niet meer dan 15 Gy, 17 Gy en 45 Gy, respectievelijk. Daar de dosisvermindering door afschermen in hoog gefractioneerde schema's relatief beperkt of soms zelf geheel afwezig kan blijven (ogen), worden hooggefractioneerde schema's beschouwd als het meest effectief.

De wijze waarop TBI in Nederland wordt toegepast verschilt aanzienlijk. Het leek daarom zinvol de resultaten in de grote Nederlandse centra voor HSCT te bekijken en uit te zoeken of er verschillen in de resultaten te vinden zijn, die teruggevoerd kunnen worden op de verschillende TBI regimes.
Om dit te bereiken werden in **Hoofdstuk 8** de BED-effect relaties van de TBI regimes die werden gebruikt als onderdeel van de conditionering voor autologe en allogene HSCT, voor AL en NHL, bestudeerd. De studie betrof 1032 patiënten, behandeld van 1990-2002. Eindpunten waren OS, RI, NRM en RFS. Om de eindpunten te evalueren werd een multivariate analyse uitgevoerd, gestratificeerd voor ziekte (AL en NHL). Voor de analyse werden de BED-waarden van de toegepaste TBI-regimes uitgerekend en verdeeld in drie dosis-groepen: BED≤14 Gy, 14<BED≤18 Gy en BED>18 Gy. Variabelen die geanalyseerd werden, behalve BED regime, waren leeftijd, geslacht en transplantatietype (autoloog of allogeen), omdat zij eventueel van invloed konden zijn geweest op de resultaten.
Ook de BED waarden voor leukemische cellen in de schaduw van de afschermende blokken werden berekend, daar de mate van afscherming van organen één van de voornaamste verschillen tussen de centra was.
Leeftijd van de patiënt en geslacht bleken de enige variabelen te zijn die, in de multivariate analyse, van significante invloed waren op de overleving. De BED-groep van de TBI

beïnvloedde de OS en RFS niet significant, maar was, voor beide ziekten, alleen van significante invloed op de NRM en de RI. We vonden geen aanwijzing dat het effect van de dosisgroep afhankelijk was van de variabelen leeftijd, geslacht, transplantatietype en ziektetype; de interactietesten hadden alle een p-waarde van >0.20.
De resultaten van onze eerdere studie (Hoofdstuk 7) konden dus slechts gedeeltelijk worden bevestigd: de RI nam wel af met toenemende dosis, maar er was geen effect van de hoogte van de TBI-dosis op de OS.
De mate van afscherming van longen en nieren leek niet van significante invloed te zijn op de incidentie van het locale recidief, noch op de OS. Reden daarvan was het lage aantal recidieven gediagnosticeerd in de schaduw van de afschermende blokken. Dit, ondanks het relatief grote volume hematopoietisch weefsel en leukemische cellen, aanwezig in het gebied met verlaagde dosis. Veel meer afwijkingen werden gevonden maar moesten worden uitgesloten wegens twijfel over de diagnose (GVHD, infectie of recidief). Verder werd, als er eenmaal verdenking bestond op een recidief, de juiste diagnose niet altijd geverifieerd als dat geen consequenties had voor de behandeling van de patiënt.
Recidieven in de schaduw van de (kleine) oogblokken waren laag in aantal, maar werden alleen gevonden als was afgedekt tot een zeer lage dosis. Zij waren ook meestal solitair. Hun voorkomen hing mogelijk samen met de extreem lage BED in de schaduw van de oogblokken.

Voor de analyse van de leeftijd van de patiënten werden drie groepen gedefinieerd: < 20 jaar, ≥ 20 en < 40 jaar en ≥ 40 jaar. We vonden een geleidelijke toename van de NRM en een afname van de RFS met toenemende leeftijd. In de multivariate analyse was invloed van de leeftijdgroepen op OS en RFS onafhankelijk significant ten gunste van jongere leeftijd, voor beide ziekten. Leeftijd had geen invloed op RI, maar een sterk significante invloed op NRM.
De HR voor NRM van patiënten > 40 jaar oud was 2.29 (p = 0.001) versus patiënten < 20 jaar. Wij konden het effect van de TBI-dosis op de RI bij kinderen, gevonden door Willemze et al., bevestigen: de lagere RI bij oudere kinderen vergeleken met jongere, is geassocieerd met de hogere TBI-dosis die oudere kinderen krijgen (25). In onze studie werd in de jongste leeftijdsgroep (<20 jaar) (aangepast voor autoloog/allogeen) hetzelfde effect van de hoogte van de TBI-dosis op de RI gevonden bij patiënten > 10 jaar vergeleken met patiënten ≤ 10 jaar. Het effect verdween bijna geheel als het model werd aangepast voor TBI-dosis.

OS en RFS van autoloog en allogeen getransplanteerde patiënten waren in de multivariate analyse niet significant verschillend. Patiënten die allogeen waren getransplanteerd hadden een significant lagere RI, maar een hogere NRM vergeleken met autoloog getransplanteerde patiënten.

Wat ziektetype betreft: patiënten met NHL hadden in univariate analyse een significant betere OS en lagere RI dan patiënten met AL.

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DANKWOORD

Dankwoord

DANKWOORD

Het avontuur is voorbij. Na veel omzwervingen is een lange weg door heuvels en diepe dalen ten einde. Er is een proefschrift.
Veel mensen zijn er bij betrokken geweest, soms lang geleden, in 1981 al, maar ook nog heel recent. Dat het onderzoek zou moeten leiden tot een proefschrift heb ik lang geprobeerd te omzeilen. Tenslotte waren zoveel inspanningen verricht door anderen en door mijzelf, dat ik niet goed meer terug kon. Maar het was de moeite waard. Ik heb veel geleerd en ook veel beleefd, vooral de laatste jaren, hoewel de laatste jaren ook moeilijk waren, omdat de weg zo eenzaam werd. Zonder de onvoorwaardelijke steun van mijn huidige en mijn vroegere co-promotor, de beide Henken, had ik het toen niet gered.

Aan het allereerste begin stond Jan Schipper met zijn enthousiasme en belangstelling voor stralingscataract. In het AZU, op de Catahrijnesingel, was beenmergtransplantatie in 1981 als behandeling van start gegaan en professor Henny van Peperzeel had Jan opgedragen een techniek voor de TBI te bedenken. Mij had ze de verantwoordelijkheid voor de patiënten toebedeeld. Jan vond de TBI stoel uit, die nog steeds in een wat modernere versie wordt gebruikt en zag meteen een nieuwe bron voor zijn hobby: onderzoek van het stralingscataract. Bedankt Jan, dat je me toen hebt gestimuleerd om daarmee te beginnen en ook bedankt dat je altijd weer zo trouw op kwam draven met het karretje met de funduscamera, op elk ongelegen moment dat ik je belde. Helaas behandelden we in het begin maar een paar patiënten per jaar, de overleving was heel matig en sommige cataracten werden pas na 6 jaar zichtbaar. Het duurde tot 1997 voordat ik voldoende gegevens had verzameld voor een artikel.
Daarna kwam alles in een stroomversnelling, nou ja, stroomversnelling, het was een vrij rustige beek, maar hij kabbelde voort en leidde tenslotte toch tot dit boekje.
Vaak denk ik ook nog aan Derk Rutgers, hij was betrokken vanaf het begin en leidde het “radiobiologie groepje” in de tijd dat ik met de eerste cataract artikelen bezig was. Hij dacht mee over onderzoek om verder te gaan en zei steevast: “En vergeet je dan niet om er een kaftje om te doen?”. Het is een mooi kaftje geworden, jammer dat Derk het niet meer kan zien.
De eerste hoofdstukken van dit proefschrift gaan over het stralingscataract en in de jaren na het vertrek van Jan Schipper werden de lensdia’s gemaakt door de fotografen van de Oogheelkunde. Zij deden het belangeloos, tussen de bedrijven door, en waren altijd

vriendelijk en bereidwillig. Dat heb ik meer op prijs gesteld dan ik ooit heb laten merken, helaas.
Natuurlijk wil ik ook graag de patiënten bedanken. Zij kwamen altijd trouw als ze, door de jaren na de transplantatie heen, steeds weer opgeroepen werden voor een lensfoto. Het was altijd boeiend om met ze te praten, zij hadden zo hun eigen kijk op behandelingen als TBI en BMT en de naweëen ervan. Zij vonden het overigens meestal geen slecht idee dat er iemand was die aandacht besteedde aan hun ogen en een mogelijk cataract.
Op de afdeling Hematologie was de lensfoto, vóórafgaand aan de BMT, tenslotte zo ingeburgerd geraakt, dat, toen het onderzoek werd gestopt, het moeite kostte de verpleegkundigen ervan te overtuigen dat een patiënt óók TBI kon krijgen als er tevoren géén lensfoto was gemaakt.

Promotor Jan Battermann, bedankt dat je de laatste jaren steeds weer een nul-aanstelling regelde, zonder veel hoop dat mijn onoverzichtelijke werk ooit zou leiden tot een proefschrift.
Promotor Ed Noordijk, bedankt, door jou zijn in het LUMC toch deuren open gegaan die anders waarschijnlijk gesloten waren gebleven.

Beste Henk Struikmans, mijn tweede promotor en vriend, toen je nog in Utrecht werkte, was je gewoon mijn collega, buurman op de afdeling en tevens selfmade co-promotor. Heel gezellig was dat toen. Na je aanstelling tot hoogleraar in Leiden, promoveerde je als het ware van co-promotor tot promotor. Zonder jou, Henk, was ik destijds helemaal niet aan de gang gebleven met dit proefschrift. Als je destijds geen regelmatige voortgangsbesprekingen had ingevoerd en nooit aflatend (ook na je vertrek naar het Westeinde) geïnteresseerd was gebleven in mijn vorderingen, had ik jaren geleden stiekem het bijltje er bij neer gegooid, zonder dat hen of haan er naar had gekraaid.

Henk Kal, mijn huidige co-promotor, aan jou ben ik het allermeeste dank verschuldigd. Je bent eerste auteur van twee artikelen in dit boekje en co-auteur van alle andere. Wat zou dit proefschrift geweest zijn zonder jouw inbreng en al jouw kennis? En dat niet alleen, je was ook letterlijk mijn steun en toeverlaat, vooral in de laatste twee jaren. Ik had het verlammende gevoel gekregen er niet meer bij te horen, mijn inspanningen leken niet echt gewaardeerd te worden, niemand vroeg meer waar ik eigenlijk mee bezig was. Het was nogal demotiverend. Maar jij was in dezelfde positie, weliswaar niet met een nul-aanstelling maar verder toch in bijna dezelfde situatie. Je werkte ook maar twee dagen per

week en jij ging onder alle omstandigheden stug door met het bedrijven van wetenschap en het produceren van een hele stroom van artikelen. Je was mijn voorbeeld en houvast: stabiel, nuchter, ijverig en toch makkelijk, héél precies en toch slordig. Eigenschappen die moeilijk te combineren lijken, maar je hebt ze allemaal. Daar kon ik toch niet bij achterblijven, NUL-aanstelling of niet. Bedankt voor alles Henk. Ik zal het na al die jaren erg gaan missen, de samenwerking, je kalme energie en die rustige kamer op het UMC. Ik hoop dat we nog lang met jou en Klaske vrienden blijven en gezellige avondjes beleven.

De professoren Hagenbeek, Theobald, Mourits, Vossen en Broerse wil ik graag hartelijk bedanken voor het zitting nemen in de beoordelingscommissie. Hoewel het wordt voorgesteld als een eer, is het waarschijnlijk voornamelijk een bezoeking een proefschrift te moeten beoordelen. Ik wil jullie allen graag van harte bedanken voor alle moeite.

Professor Jaak Vossen wil ik graag ook nog bedanken voor zijn hulp bij het opzetten van het onderzoek naar het effect van oogblokken bij kinderen in Leiden (en Utrecht). De inspanning om een student (Carolien Emmens) te vinden die kon helpen met het status onderzoek, ik was toen nog niet met pensioen, en ook je trouwe en vriendelijke aanwezigheid bij de voortgangsbesprekingen, die altijd heel gezellig waren, ik heb het erg gewaardeerd. Ook daarna hebben we nogal eens contact gehouden. Jammer dat zulke dingen voorbij gaan.

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artikel zonder die vaardigheid? Onze open haard gaat ook heel mooi worden. Je bent erg veelzijdig, dat is een ding dat zeker is.

Mijn collega's bedankt voor de belangstelling en een praatje zo af en toe, vooral de "oude garde" Harm, Chris, Carla, die vrienden zijn geworden, waar natuurlijk ook Henk Struikmans en Babs Reichgelt bijhoren. Ook Marco, Robert, Homan, en andere "ganggenoten", bedankt voor de kameraadschap, vriendelijkheid en belangstelling.
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Beste ganggenoot professor Jan Lagendijk, bedankt dat ik altijd bij je binnen kon lopen, voor een praatje en advies vanwege je grote ervaring met promoties en promovendi.
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waar je weer een professionele en magnifieke hoofdrol in vervulde. Jou beschouw ik een beetje als mijn radiotherapie-dochter en ik ben heel trots op je!

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Dit proefschrift is speciaal opgedragen aan mijn vader en aan Vera en wil een monument, een gedenkteken voor hen zijn. Het idee dat het een monument moest worden was zelfs één van de redenen om door te zetten met het werk. Aan Rosa en Martje is het opgedragen omdat zij de toekomst hebben. Ik hoop dat het hen helpen zal te beseffen hoe belangrijk zij zijn en dat het er op aan komt uit te groeien tot een zelfstandige vrouw en man die hun gaven gebruiken en uit het leven halen wat mogelijk is. Mijn vader heeft kort voordat hij vertrok iets dergelijks tegen mij gezegd. Ik was heel jong toen, maar ik ben het nooit vergeten.
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Tenslotte komt toch aan alles een eind, in dit geval zelfs aan veel zaken tegelijk: proefschrift, wetenschap, radiotherapie toepassen, rondlopen en thuis zijn in het UMC en wonen in de provincie Utrecht. Er breekt een heel nieuwe tijd aan met nieuwe

mogelijkheden: parttime filosofie studeren in Rotterdam b.v. en portrettekenen verder verbeteren, misschien weer gaan schrijven en veel en héél ver langs het strand lopen met de honden, om maar wat te noemen. Op naar de toekomst!

Dankwoord

CURRICULUM VITAE

Curriculum

CURRICULUM VITAE

Loes Harteveld werd op 16 oktober 1939 in Den Haag geboren.
In 1959 behaalde zij het diploma gymnasium B aan het Tweede Vrijzinnig Christelijk Lyceum in Den Haag.
In september 1959 startte zij met de studie geneeskunde aan de universiteit van Leiden. In 1960 behaalde zij het eerste deel van het kandidaatseaxamen. In 1961 besloot zij met de studie te stoppen en werkte zij een tijd lang als analiste en medisch secretaresse op de afdeling Hematologie van het Academisch Ziekenhuis in Leiden.
In 1963 trouwde zij en kreeg twee kinderen (1965 en 1966).
In 1969, nadat het gezin was verhuisd naar Vleuten-De Meern (waarvoor was gekozen i.v.m. de nabijheid van een universiteit), hervatte zij de medische studie aan de universiteit van Utrecht. De studieopzet in Utrecht was inmiddels drastisch gewijzigd, wat betekende dat de studie vrijwel opnieuw gestart moest worden, afgezien van vrijstelling voor de helft van de tentamens in het eerste jaar.
Na het artsexamen in 1977 werkte zij vanaf 1 januari 1978 als arts-assistent in opleiding op de afdeling Radiotherapie van het Academisch Ziekenhuis in Utrecht, (opleider Prof. dr. H.A. van Peperzeel). In 1980 was zij gedurende 9 maanden werkzaam als asssistent in opleiding op de afdeling Nucleaire Geneeskunde, maar hervatte de opleiding op de afdeling Radiotherapie in februari 1981.
Vanaf 1 oktober 1984 was zij lid van de medische staf op dezelfde afdeling.
In 2001 ging zij vervroegd met pensioen en kreeg aansluitend een ‘nulaanstelling’ (die jaarlijks werd verlengd) voor het verrichten van medisch wetenschappelijk onderzoek.
In 2005 werkte zij 7 maanden als waarnemend radiotherapeut in het Hagaziekenhuis, locatie Leyenburg in Den Haag.
Vanaf mei 2006 tot 2011 is zij geherregistreerd als radiotherapeut op grond van gelijkgestelde werkzaamheden (wetenschappelijk onderzoek op een relevant gebied van de geneeskunde).
Loes is getrouwd met Ruud van Kempen en samen hebben zij twee zoons, Michiel en Wouter, en drie kleinkinderen, Rosa, Vera† en Martijn.


Ik ben een oude rups –
Die verhalen over een vlinder,
die gebeuren toch nooit!
Soulas