Late Effects After Autologous Bone Marrow Transplantation in Childhood

BY

PER FRISK

ACTA UNIVERSITATIS UPSALIENSIS
UPPSALA 2003
Dissertation presented at Uppsala University to be publicly examined in Rosénsalen, Akademiska barnsjukhuset, 751 85 Uppsala, Friday, November 21, 2003 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

Fifty children with hematologic malignancies have been treated with autologous BMT in Uppsala. The aim was to describe late effects in this group with special reference to cataracts, reduced final height, and to hepatic, renal, and pulmonary late effects.

Cataracts: All patients who received TBI in their conditioning developed posterior subcapsular cataract after BMT. A few patients with visual impairment affecting daily life needed cataract surgery, whereas the visual acuity was well preserved in most of the other patients.

Final height: There was a decrease in final height relative both to height at BMT and to target height. This decrease was significant both in those who had received TBI only and in those who had been given cranial irradiation. Cranial irradiation, young age at BMT, and short duration of GH treatment were predictors of height loss.

Hepatic function: Hepatic function was well preserved over a period of 10 years after BMT. TBI did not appear to be a risk factor for hepatic impairment.

Renal function: Six months after BMT there was a decrease in renal function in patients who had received TBI. It then recovered, albeit incompletely, and stabilized. After the first year there was little change over a period of 10 years after BMT. TBI appeared to be the most important risk factor for the development of chronic renal impairment in a number of patients. Nephrotoxic antibiotics may have contributed.

Pulmonary function: Six months after BMT there was a decrease in pulmonary function in those who received TBI. It then recovered and stabilized at the pretransplant level. After the first year there was little change over a period of 10 years after BMT. TBI appeared to be the most important risk factor for restrictive pulmonary disease in a number of patients whereas chemotherapy might also have been of importance for impaired gas exchange.

Keywords: bone marrow transplantation, children, follow-up, cataracts, final height, pubertal development, hepatic function, renal function, pulmonary function

Per Frisk, Department of Women's and Children's Health, Uppsala University, SE-75185 Uppsala, Sweden

© Per Frisk 2003

ISSN 0282-7476
ISBN 91-554-5767-3
urn:nbn:se:uu:diva-3673 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-3673)
List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


II. Frisk, P., Arvidson, J., Gustafsson, J., Lönnerholm, G. Pubertal development and final height after autologous bone marrow transplantation. *Bone Marrow Transplantation* (accepted)


Reprints were made with the permission of the publishers.
Hepatic impairment before BMT .................................................... 25
Acute hepatic impairment after BMT ............................................. 25
Chronic hepatic impairment after BMT ............................................ 26
Renal function after autologous BMT (paper IV) ............................. 29
Introduction and rationale for the study ............................................ 29
Risk factors for renal impairment after autologous BMT ...... 29
Additional risk factors for renal impairment after allogeneic BMT 29
Patients and methods ........................................................................ 30
Definitions ....................................................................................... 30
Results and discussion ........................................................................ 30
Renal impairment before BMT ....................................................... 30
Acute renal impairment after BMT ................................................. 30
Chronic renal impairment after BMT ............................................. 31
Pulmonary function after autologous BMT (paper V) ....................... 37
Introduction and rationale for the study ............................................ 37
Risk factors for pulmonary impairment after autologous BMT .... 37
Additional risk factors for pulmonary impairment after allogeneic
BMT .............................................................................................. 37
Idiopathic pneumonia syndrome ................................................. 37
Patients and Methods ...................................................................... 38
Definitions ....................................................................................... 38
Results and discussion ...................................................................... 38
Pulmonary impairment before BMT ................................................. 38
Early pulmonary complications .................................................... 38
Impaired pulmonary function tests ............................................ 39
  Restrictive impairment .............................................................. 39
  Obstructive impairment ............................................................ 41
  Isolated diffusing impairment .................................................... 41
Respiratory symptoms .................................................................... 43
Body proportions ............................................................................. 43
Conclusions and clinical implications .................................................. 44
Paper I ............................................................................................. 44
Paper II ............................................................................................ 44
Paper III ........................................................................................... 45
Paper IV ........................................................................................... 45
Paper V ............................................................................................ 45
Future aspects .................................................................................. 47
Abbreviations

ALL Acute lymphoblastic leukemia
ALT Alanine aminotransferase
AST Aspartate aminotransferase
ALP Alkaline phosphatase
AML Acute myeloblastic leukemia
ANOVA Analysis of variance
BMT Bone marrow transplantation
BMT Np Bone marrow transplantation nephropathy
BCNU 1-3 bis chloroethyl-1 nitrosurea
BU Busulfan
CMV Cytomegalovirus
CNS Central nervous system
CI Cranial irradiation
CR Complete remission
Cy Cyclophosphamide
$^{51}$Cr-EDTA $^{51}$Chromium-labeled ethylenediaminetetra-acetic acid
DLCO The diffusing capacity of the lung for carbon monoxide
DMSO Dimethylsulphoxide
CO Carbon monoxide
EBV Epstein-Barr virus
ECCE Extracapsular cataract extraction
ERPF Effective renal plasma flow
FEF$_{25}$ Forced expiratory flow at 25% of FVC
FEF$_{50}$ Forced expiratory flow at 50% of FVC
FEF$_{75}$ Forced expiratory flow at 75% of
FVC

FEV₁

FEV₁/VC

FF

FRC

FSH

FTBI

GH

GHD

GFR

GnRH

GVHD

Gy

HAV

HbsAg

HCV

HD

HLA

H-SDS

HUS

IgG

IgM

131IOH

IOL

IPS

LBL

LH

LCAL

MEF

NHL

NOPHO

p-EFS

PCR

PFT

PSC

RV

Forced expiratory volume in one second
FEV₁ in percent of VC
Filtration fraction
Functional residual capacity
Follicle stimulating hormone
Fractionated total body irradiation
Growth hormone
Growth hormone deficiency
Glomerular filtration rate
Gonadotrophin releasing hormone
Graft-versus-host disease
Gray
Hepatitis A virus
Hepatitis B surface antigen
Hepatitis C virus
Hodgkin’s disease
Human leukocyte antigen
Height standard deviation score
Hemolytic-uremic syndrome
Immunoglobulin G
Immunoglobulin M
131Iodine-labeled hippurate
Intraocular lens
Idiopathic pneumonia syndrome
Lymphoblastic lymphoma
Luteinizing hormone
Large cell anaplastic lymphoma
Maximum expiratory flow
Non-Hodgkin’s lymphoma
The Nordic Society of Pediatric Hematology and Oncology
Probability for event-free survival
Polymerase chain reaction
Pulmonary function test
Posterior subcapsular cataract
Residual volume
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPSS</td>
<td>Statistical package for the social sciences</td>
</tr>
<tr>
<td>TBI</td>
<td>Total body irradiation</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>TH</td>
<td>Target height</td>
</tr>
<tr>
<td>VA</td>
<td>Visual acuity</td>
</tr>
<tr>
<td>VC</td>
<td>Vital capacity</td>
</tr>
<tr>
<td>VOD</td>
<td>Veno-occlusive disease</td>
</tr>
</tbody>
</table>
Background

Introduction

During recent decades there has been a marked improvement in survival in children with cancer. Increasing attention is therefore being focused on issues relating to late effects of cancer therapy with the aim to facilitate therapeutic modifications that will minimize them.

Current estimations indicate that more than 30000 allogeneic and autologous bone marrow transplantations are performed each year worldwide. Approximately one-fifth are performed in children and it is estimated that annually a minimum of 1500-2000 of these will become long-term survivors. [1]

The description of late effects after autologous bone marrow transplantation (BMT) is the main topic of this thesis. When evaluating late effects of therapy in a transplant population consideration needs to be given not only to the transplant-related therapy but also to the disease and its primary treatment, which in our population included acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML), Non-Hodgkin’s lymphoma (NHL), and Hodgkin’s disease (HD).

Epidemiologic data presented here are derived from the latest report issued by The Nordic Society of Pediatric Hematology and Oncology (NOPHO). [2] NOPHO was established in 1981 and a registry was created early for the co-ordination of diagnostic procedures, treatment, and follow-up of childhood malignancies within the Nordic region. Since 1995, joint reports from registries and from various working groups concerned with special therapeutic topics have been presented annually.

Acute lymphoblastic leukemia

ALL is the most common cancer in children and constitutes about 30% of all childhood malignancies. [2] The annual incidence between 1981 and 1998 was 3.9/100000 children below 15 years of age in the Nordic countries. [3] The overall probability of event-free survival (p-EFS) was 0.74 for the time period 1992-2001. [2] The most recent NOPHO treatment protocol was
launched in 2000, but patients treated according to this protocol are still too few for any conclusions to be drawn on changes in outcome.

Treatment of ALL has traditionally been based on stratification according to risk groups (standard risk, intermediate risk, high-risk, and very high-risk) which in turn are based on certain risk criteria, such as age and tumor burden at diagnosis, involvement of the central nervous system (CNS) or of the testes, immunophenotype and karyotype of the lymphoblasts, and response to the induction treatment. Therapy in the NOPHO treatment protocols is divided into four main phases: induction therapy, CNS-directed therapy, late intensification (not in standard risk patients), and maintenance therapy. CNS-directed treatment previously consisted of cranial irradiation (CI) in intermediate and high-risk patients (in almost 60% of patients). Since 1992 this treatment has been substituted with intravenous infusions of high-dose methotrexate in combination with intrathecal injections of methotrexate. Today, only patients considered very high-risk and patients with overt CNS involvement are given CI (less than 10% of patients). [3]

Relapse occurs in 25-30% of patients. [4] There is no defined ALL relapse protocol within NOPHO, but the NOPHO high-risk protocol or the relapse protocol of the German BFM-group have primarily been used, including irradiation to the testes and the CNS in patients with overt testicular or CNS relapse. Today, the best postremission therapy for high-risk ALL and for relapsed ALL is considered to be allogeneic BMT. [5, 6]

Late effects of ALL treatment include hypogonadism induced by alkylating agents and cardiac impairment induced by anthracyclines. Cranial irradiation increases the risk of secondary CNS tumors, neurocognitive deficits, hypothyroidism, precocious puberty, growth hormone deficiency, short adult stature, obesity, dental abnormalities, and cataracts. [7]

Acute myeloblastic leukemia

AML is rarer and carries a poorer prognosis than ALL. It constitutes about 5% of all childhood malignancies. [2] The annual incidence is 0.7/100000 in children. Children with Down’s syndrome accounted for 13% of these cases. The p-EFS was 0.81 for children with Down’s syndrome and 0.43 for other children for the time period 1993-2002.

Remission is achieved in about 85% of children, but without intensive consolidation therapy consisting of intensive chemotherapy or BMT, most children will relapse. The best consolidation therapy is considered to be allogeneic BMT. [8] The present view is that autologous BMT offers no substantial advantage over chemotherapy as treatment in first remission. [8, 9]

In comparison with ALL, the treatment of AML is characterized by higher doses of anthracyclins, shorter duration of treatment, and less frequent
use of cranial irradiation. In the largest report to date, major late effects of therapy not using BMT included secondary tumors and cardiac impairment. [10]

Non-Hodgkin’s lymphoma

NHL (also including Burkitt’s lymphoma and unspecified lymphomas) constitutes about 5% of all childhood malignancies. [2] The p-EFS was 0.85 in patients diagnosed in 1995-2002.

The most widely used chemotherapy regimens for lymphoblastic lymphoma (LBL) are based on protocols designed for ALL. Patients with relapsed NHL should be considered for autologous BMT. [11]

The use of radiotherapy has decreased over the years. Late effects of chemotherapy include cardiotoxicity induced by anthracyclines, hypogonadism induced by alkylating agents, and secondary tumors. [12]

Hodgkin’s disease

HD constitutes about 4% of all childhood malignancies. [2] The p-EFS was 0.96 in patients diagnosed 1985-1994.

Therapy is based on combined modality therapy that has allowed a reduction in both radiotherapy and chemotherapy. Patients with relapsed HD should be considered for autologous BMT [13]

Late effects of chemoradiotherapy include growth impairment, hypogonadism, hypothyroidism, cardiac and pulmonary impairment, and secondary tumors, especially breast cancer in females receiving radiotherapy against the chest. [14]

Bone marrow transplantation

In 1951, Lorentz et al reported that mice given supralethal irradiation could be protected by an infusion of spleen (a hematopoetic organ in the mice) and marrow cells. [15] In 1956, Barnes et al reported the treatment of leukemic mice by supralethal irradiation followed by infusion of normal mouse marrow. [16] In 1957, Thomas et al showed that bone marrow could be infused and result in a transient engraftment in man, and five years later, Mathé et al achieved the first persistent allogeneic marrow graft in a patient with leukemia. [17, 18] In 1977, Thomas et al reported the results from the first 100 patients with advanced acute leukemia who were prepared with cyclophosphamide and TBI and given marrow from a HLA-matched sibling. [19]
Today, bone marrow transplantation (BMT) has evolved into an established treatment for hematological malignancies in high-risk patients who are known to have a poor prognosis with conventional chemotherapy. In order to eradicate remaining malignant cell clones and to create space for the stem cells to be infused (and in the case of allogeneic grafts also to suppress the immunological resistance of the recipient to allow engraftment), the patient undergoes intensive conditioning with high doses of cytostatic agents and often with total body irradiation. The depleted bone marrow is then restored by infusion of previously collected healthy bone marrow. Without this restoration, the patient would succumb to the consequences of marrow aplasia, including infection, bleeding, and profound anemia.

There are three principal types of bone marrow transplantation. In autologous BMT the patient's own bone marrow, previously collected and stored after ascertainment of clinical remission, is re-infused. In syngeneic BMT the donor and recipient are monozygous twins. In allogeneic BMT the patient receives bone marrow from a histocompatible donor. Compatibility is determined by the matching of the human leukocyte antigen (HLA) system which is made up of proteins in the cell membranes that function normally in antigen recognition of foreign agents and are important in immunologic recognition of foreign tissues.

A major complication after allogeneic BMT is the so-called graft-versus-host disease (GVHD), a reaction where immunocompetent cells in the donor marrow react against the recipient. Acute GVHD develops within 3 months after allogeneic BMT and affects mainly the skin, the liver, and the gastrointestinal tract. [20] Chronic GVHD occurs by definition 100 days after BMT. It is usually preceded by acute GVHD but may also occur de novo. Chronic GVHD mimicks in many ways autoimmune disorders and is clinically characterized by dermatitis, keratoconjunctivitis, oral mucositis, generalised sicca syndrome, and hepatic and pulmonary impairment. [21] Chronic GVHD is also associated with a sustained impairment of the host immunity that may predispose to infections. There is evidence of an antitumoral effect of GVHD itself, the so-called graft-versus-leukemia effect. [22] Prophylaxis against GVHD consists of cyclosporine A alone or in combination with methotrexate. [23] Cyclosporine A is mainly limited by its nephrotoxicity. [24]

The most desirable donor in allogenic BMT is an HLA-identical sibling donor. If a BMT-candidate lacks an HLA-identical sibling donor, one may instead use an HLA-mismatched family donor, an HLA-identical unrelated donor, or perform autologous BMT. One advantage of autologous BMT is that it precludes the need to search for suitable donors. Another advantage is the absence of GVHD, resulting in modest peri-transplant morbidity and mortality. [25] These advantages have to be considered in relation to a certain risk of re-infusion of malignant cells and a theoretical inferior antitumoral effect of autologous BMT through the lack of the graft-versus-
leukemia effect. Consequently, relapse is by far the most common cause of treatment failure after autologous BMT, nonrelapse mortality being low, whereas the opposite is true of allogeneic BMT. [25] During recent years the number of allogeneic BMTs has increased at the expense of autologous BMT in the treatment of childhood leukemia. This is a result of new molecular techniques which have made it easier to find suitable donors in the expanding voluntary donor registers, as well as improvements in the treatment of GVHD and its sequelae. [26]

It has proved exceedingly difficult to perform randomized controlled trials in the field of BMT and decision-making is therefore based on largely inconclusive studies. The optimal treatment for patients who are known to have a poor prognosis with conventional chemotherapy is therefore continuously being debated and BMT indications will vary with time. Today, the major indications for autologous BMT in pediatric hematologic malignancies are progressive or relapsed lymphomas, whereas high-risk or relapsed leukemias are preferably treated with allogeneic BMT.

Finally, a note on semantics. Bone marrow is the traditional source of hematopoietic stem cells. In recent years peripheral blood progenitor cells and umbilical cord blood cells have increasingly been used. The terminology has therefore shifted from bone marrow transplantation to hematopoietic stem cell transplantation (HSCT). It could, furthermore, be argued that re-infusion of the patient’s own cells (as in autologous HSCT) does not constitute proper transplantation. Since the present thesis in many ways is based on previous work by the Uppsala BMT group we have decided, in order to avoid confusion, to retain the traditional terminology.

Total body irradiation

TBI is added to the conditioning regimen prior to BMT in order to kill widely dispersed radiosensitive cells and reaches sites that are poorly penetrated by cytostatic agents, such as the testicles and the CNS (so-called sanctuary sites). All organ systems in the body will be damaged by irradiation, although the effects on slowly proliferating cell populations may emerge only with time. In order to decrease late effects, various strategies have been introduced, such as fractionation of the radiation dose and shielding of vital organs. [27, 28] Fractionation of TBI allows tissues with large repair capacity time to repair between fractions, whereas tissues with little repair capacity, such as bone marrow stem cells, will succumb. In a prospective randomized clinical trial comparing single dose TBI with fractionated TBI, survival was significantly better in the fractionated group due to lower treatment-related mortality, whereas the rate of leukemic relapse was equal in both groups. [29]
Many centers also attempt to reduce late effects by using preparative regimens without TBI. A prospective randomized clinical trial conducted by the Nordic Bone Marrow Transplantation Group comparing patients conditioned with either TBI or busulfan in addition to cyclophosphamide reported a superior survival with TBI, especially in patients with advanced disease. [30] A pediatric study found a similar advantage of TBI over busulfan-containing regimens in children with ALL due to the lower treatment-related mortality with TBI. [31] Consequently, late effects related to TBI will still be encountered in many long-term survivors in the near future.

In a recent review of late effects after BMT, Socié et al stated that TBI together with chronic GVHD and/or its treatment were the major risk factors for nonmalignant complications after BMT. [32] The absence of chronic GVHD and its treatment in autografted patients may permit the elucidation of the independent role of TBI after BMT.

Late complications of bone marrow transplantation

The aggressive nature of cancer therapy may damage normal tissue and so affect the function of vital organs. Such consequences are especially severe in children, as developing tissue is highly susceptible to damage. The younger the patient, the greater the potential for late effects of treatment. Important late sequelae of BMT in childhood not reported in the current thesis, include neuropsychologic sequelae, cardiac impairment, and secondary malignancies. Neuropsychologic sequelae are particularly common in children given TBI before the age of 3 years, and in those who have been previously exposed to cranial irradiation in addition to TBI. Risk factors for cardiac impairment include previous anthracycline usage, chest irradiation, and cyclophosphamide. The most commonly reported secondary malignancies are post-transplant lymphoproliferative disorders and solid tumors. Thyroid and brain tumors appear to be the most common solid tumors after BMT, particularly in young children and in those who have been previously exposed to cranial irradiation in addition to TBI. ALL occurring in donor cells after allogeneic BMT, and myelodysplasia and AML, mainly associated with autologous BMT, are uncommon. [32-36]

Scientific considerations

Major strengths of the studies that make up this thesis include the longitudinal design, the long-term follow-up, the small loss to follow-up, and the high validity and reliability of the measurements. The studies are not without limitations, however. The patients were enrolled during a long
period of time (from 1985 to 1997), exposing the sample to secular trends of therapy, including, for example, changes of the primary treatment before BMT, of the TBI regimen, and of the supportive care after BMT. In consequence, patients transplanted at an early date may not be directly comparable with those transplanted later. Furthermore, there is an intrinsic association between, for example, age at BMT, diagnosis, and preparative regimen in our population that may cause difficulty when attempting to ascertain causation. These potential biases are compounded by the low power of the statistical analyses due to the small number of patients.

Throughout the thesis our results are compared with those of other studies. Here, it is important to keep in mind the great heterogeneity between reported series which make comparisons notoriously difficult. Our results are based on children who have undergone autologous BMT using therapies peculiar to Uppsala and cannot therefore be directly generalized to other BMT populations. However, with full awareness of these problems, we believe that our results may provide a basis for the understanding of late effects after autologous BMT, as well as, mutatis mutandi, for other BMT modalities, and for comparisons between them. Specifically, our results may provide a basis for physicians' recommendations and information to the patients and their families, and for the design of future follow-up programs. Without chronic GVHD and its treatment as confounders, late effects of TBI may be easier to assess.
Aims of the studies

The principal aim of the present studies was to describe late effects in a group of long-term survivors of childhood leukemias and lymphomas in whom the treatment included autologous BMT. The aims of the studies were:

- to describe the development and treatment of cataracts with or without TBI.
- to describe pubertal development and final height in ALL patients who received TBI.
- to describe GH status and the effect of GH treatment in ALL patients who received TBI.
- to describe the long-term development of hepatic function with or without TBI.
- to describe the long-term development of renal function as measured by $^{51}$Cr-EDTA, $^{131}$I OH clearance, and the desmopressin test, with or without TBI.
- to describe the long-term development of pulmonary function with or without TBI.
Patients and Methods

Patients
Between October 1985 and August 1997, 50 patients younger than 18 years of age were treated with autologous BMT (n=49) or syngeneic BMT (n=1) at the University Hospital of Uppsala, which serves as a tertiary referral center for pediatric oncology. Children with ALL who lacked a matched sibling donor were autografted in first complete remission in very high-risk cases after individual assessment, and in second remission after a bone marrow relapse. Patients with extramedullary relapse were autografted if they had relapsed on therapy or within 6 months of discontinued therapy. These patients also received cranial or testicular irradiation. [37] Children with AML were autografted in first complete remission if they lacked a matched sibling donor. Also, children with progressive or relapsed lymphomas were candidates for autologous BMT. No transplant-related deaths occurred and procedure-related morbidity was low. Nineteen patients relapsed during follow-up, with a median time to relapse of 6 months (range 2-48 months). One girl with AML had a second malignancy (Ewing sarcoma) 60 months after BMT. She is still alive but has been excluded from the follow-up.

All patients were included in the follow-up program which lasts until final height has been reached and at least for 10 years after BMT. Patients were seen before BMT, 3, 6, 9, and 12 months after BMT, and then annually. Forty patients survived more than 6 months after BMT and constitute our principal group of study. Of these, nine patients relapsed with a median time to relapse of 15 months (range 7 to 48 months) and thirty-one patients are currently alive. Median long-term follow-up is 10 years. Baseline data of the 40 patients who were followed more than 6 months and are included in any of the five papers that constitute the thesis are summarized in Table 1. The loss to follow-up has been very low. One boy transferred his follow-up to the local university hospital (Lund) and the results of his examinations have been forwarded to Uppsala. One boy transferred his follow-up to the local university hospital (Linköping) from but from 5 years after BMT he abstained from regular visits. He returned to our attention 8 years after BMT at the age of 18.5 years and underwent a limited endocrinological assessment.
Table 1 Characteristics of the 40 BMT patients studied in any of the presented papers, stratified according to age at diagnosis, TBI and previous CNS irradiation.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Diagnosis</th>
<th>Age at diagnosis</th>
<th>CI (Gy)</th>
<th>Remission status</th>
<th>Age at BMT</th>
<th>TBI Current status</th>
<th>Years since BMT</th>
<th>Included in paper No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boy</td>
<td>AML</td>
<td>1.4</td>
<td>0</td>
<td>1</td>
<td>1.9</td>
<td>0</td>
<td>CR</td>
<td>I,III,IV,V</td>
</tr>
<tr>
<td>Girl</td>
<td>AML</td>
<td>4.6</td>
<td>0</td>
<td>1</td>
<td>5.1</td>
<td>0</td>
<td>CR</td>
<td>6.4</td>
</tr>
<tr>
<td>Girl</td>
<td>AML</td>
<td>5.2</td>
<td>0</td>
<td>1</td>
<td>5.8</td>
<td>0</td>
<td>R-15</td>
<td>III,IV</td>
</tr>
<tr>
<td>Boy</td>
<td>AML</td>
<td>5.8</td>
<td>0</td>
<td>1</td>
<td>6.5</td>
<td>0</td>
<td>CR</td>
<td>12.5</td>
</tr>
<tr>
<td>Girl</td>
<td>NHL</td>
<td>6.7</td>
<td>0</td>
<td>2</td>
<td>7.9</td>
<td>0</td>
<td>SM-60</td>
<td>III,IV</td>
</tr>
<tr>
<td>Boy</td>
<td>AML</td>
<td>8.3</td>
<td>0</td>
<td>1</td>
<td>9.2</td>
<td>0</td>
<td>R-7</td>
<td>III,IV</td>
</tr>
<tr>
<td>Boy</td>
<td>NHL</td>
<td>11.9</td>
<td>0</td>
<td>2</td>
<td>12.7</td>
<td>0</td>
<td>CR</td>
<td>15.1</td>
</tr>
<tr>
<td>Girl</td>
<td>HD</td>
<td>12.5</td>
<td>0</td>
<td>2</td>
<td>14.6</td>
<td>0</td>
<td>R-24</td>
<td>III,IV</td>
</tr>
<tr>
<td>Boy</td>
<td>AML</td>
<td>12.9</td>
<td>0</td>
<td>1</td>
<td>13.7</td>
<td>0</td>
<td>CR</td>
<td>15.8</td>
</tr>
<tr>
<td>Girl</td>
<td>AML</td>
<td>13.4</td>
<td>0</td>
<td>1</td>
<td>14.0</td>
<td>0</td>
<td>R-12</td>
<td>III,IV</td>
</tr>
<tr>
<td>Boy</td>
<td>AML</td>
<td>14.5</td>
<td>0</td>
<td>1</td>
<td>15.2</td>
<td>0</td>
<td>CR</td>
<td>11.6</td>
</tr>
<tr>
<td>Boy</td>
<td>HD</td>
<td>14.9</td>
<td>0</td>
<td>2</td>
<td>17.8</td>
<td>0</td>
<td>CR</td>
<td>13</td>
</tr>
<tr>
<td>Boy</td>
<td>AML</td>
<td>15.2</td>
<td>0</td>
<td>1</td>
<td>15.7</td>
<td>0</td>
<td>R-15</td>
<td>III,IV</td>
</tr>
<tr>
<td>Boy</td>
<td>HD</td>
<td>16.2</td>
<td>0</td>
<td>2</td>
<td>17.9</td>
<td>0</td>
<td>CR</td>
<td>11.3</td>
</tr>
<tr>
<td>Girl</td>
<td>ALL</td>
<td>1.2</td>
<td>0</td>
<td>2</td>
<td>4.6</td>
<td>1</td>
<td>CR</td>
<td>18.1</td>
</tr>
<tr>
<td>Girl</td>
<td>ALL</td>
<td>1.8</td>
<td>0</td>
<td>2</td>
<td>7.0</td>
<td>1</td>
<td>CR</td>
<td>16.2</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>2.3</td>
<td>0</td>
<td>2</td>
<td>5.2</td>
<td>1</td>
<td>CR</td>
<td>16.7</td>
</tr>
<tr>
<td>Girl</td>
<td>ALL</td>
<td>2.8</td>
<td>0</td>
<td>2</td>
<td>7.4</td>
<td>1</td>
<td>CR</td>
<td>13.4</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>2.9</td>
<td>0</td>
<td>1</td>
<td>3.6</td>
<td>1</td>
<td>CR</td>
<td>17.5</td>
</tr>
<tr>
<td>Girl</td>
<td>ALL</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>4.7</td>
<td>1</td>
<td>R-9</td>
<td>III,IV</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>3.7</td>
<td>0</td>
<td>2</td>
<td>9.8</td>
<td>1</td>
<td>CR</td>
<td>17.1</td>
</tr>
<tr>
<td>Boy</td>
<td>NHL</td>
<td>4.2</td>
<td>0</td>
<td>2</td>
<td>5.8</td>
<td>1</td>
<td>CR</td>
<td>9.1</td>
</tr>
<tr>
<td>Girl</td>
<td>ALL</td>
<td>4.2</td>
<td>0</td>
<td>2</td>
<td>9.0</td>
<td>1</td>
<td>CR</td>
<td>16.5</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>5.3</td>
<td>0</td>
<td>2</td>
<td>8.6</td>
<td>1</td>
<td>R-9</td>
<td>III,IV</td>
</tr>
<tr>
<td>Boy</td>
<td>NHL</td>
<td>6.6</td>
<td>0</td>
<td>2</td>
<td>7.2</td>
<td>1</td>
<td>CR</td>
<td>9.8</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>7.4</td>
<td>0</td>
<td>2</td>
<td>10.3</td>
<td>1</td>
<td>CR</td>
<td>9.6</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>7.8</td>
<td>0</td>
<td>2</td>
<td>12.5</td>
<td>1</td>
<td>CR</td>
<td>17</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>9.2</td>
<td>0</td>
<td>1</td>
<td>10.2</td>
<td>1</td>
<td>CR</td>
<td>15.6</td>
</tr>
<tr>
<td>Girl</td>
<td>ALL</td>
<td>9.2</td>
<td>0</td>
<td>2</td>
<td>14.2</td>
<td>1</td>
<td>CR</td>
<td>14.2</td>
</tr>
<tr>
<td>Girl</td>
<td>ALL</td>
<td>2.2</td>
<td>19.5</td>
<td>3</td>
<td>5.6</td>
<td>1</td>
<td>CR</td>
<td>15.9</td>
</tr>
<tr>
<td>Girl</td>
<td>ALL</td>
<td>2.9</td>
<td>24</td>
<td>2</td>
<td>9.7</td>
<td>1</td>
<td>CR</td>
<td>16.5</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>3.3</td>
<td>24</td>
<td>2</td>
<td>6.7</td>
<td>1</td>
<td>CR</td>
<td>13</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>4.2</td>
<td>21</td>
<td>2</td>
<td>8.3</td>
<td>1</td>
<td>R-48</td>
<td>III,IV</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>4.6</td>
<td>18</td>
<td>2</td>
<td>7.7</td>
<td>1</td>
<td>CR</td>
<td>13.6</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>4.8</td>
<td>18</td>
<td>2</td>
<td>8.3</td>
<td>1</td>
<td>CR</td>
<td>13.9</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>4.8</td>
<td>18</td>
<td>2</td>
<td>10.0</td>
<td>1</td>
<td>CR</td>
<td>12.8</td>
</tr>
<tr>
<td>Girl</td>
<td>ALL</td>
<td>6.6</td>
<td>26</td>
<td>2</td>
<td>13.5</td>
<td>1</td>
<td>CR</td>
<td>17.8</td>
</tr>
<tr>
<td>Boy</td>
<td>ALL</td>
<td>10.6</td>
<td>18</td>
<td>2</td>
<td>13.1</td>
<td>1</td>
<td>CR</td>
<td>10.4</td>
</tr>
<tr>
<td>Girl</td>
<td>ALL</td>
<td>10.7</td>
<td>24</td>
<td>2</td>
<td>13.9</td>
<td>1</td>
<td>R-21</td>
<td>III,IV</td>
</tr>
<tr>
<td>Boy</td>
<td>NHL</td>
<td>15</td>
<td>21</td>
<td>2</td>
<td>17.7</td>
<td>1</td>
<td>CR</td>
<td>15.5</td>
</tr>
</tbody>
</table>

CR=complete remission; R-15=relapse 15 months after BMT; SM-60=second malignancy 60 months after BMT (Ewing’s sarcoma)
Methods

Handling of the bone marrow

Bone marrow (1-5 x 10^8 nucleated cells/kg body weight) was harvested from the posterior iliac crest with the child in general anesthesia and stored with 10% dimethylsulfoxide (DMSO) at –196°C. For ALL patients, the marrow was purged using cytolytic monoclonal antibodies plus rabbit complement. After thawing, the marrow was reinfused through a central vein on day 0. [38]

Conditioning regimens

Patients with ALL and LBL were conditioned with prednisolone 100 mg/m² orally on days -6 and -5; teniposide 200 mg/m² iv; daunorubicin 30 mg/m² iv; vincristine 1.5 mg/m² (max 2 mg) iv on day -6; cytarabine 500 mg/m² iv on days -6 to -2, and cyclophosphamide 40 mg/m² iv on days -4 and -3, plus TBI. In most patients TBI was given in a single fraction on day -1 as two opposed 5 MV X-ray anterior-posterior fields with lung shielding. The TMS dose planning system was used to calculate the dose to the patient and the thickness of the lung blocks made of Rose’s metal, which were applied when the posterior field was treated. If the measured dose to a part of the body deviated by > 5% from the dose to the normalization point, a compensating filter or an extra dose was added. The total absorbed dose in the center of the patient was 7.5 Gy (dose rate 15 cGy/min), and the maximum dose to the lungs and kidneys was also 7.5 Gy ± 5%. The four patients most recently undergoing transplantation were treated with fractionated TBI, consisting of 12 Gy in six fractions over 3 days (dose rate 15 cGy/min).

Patients with AML received busulfan 1 mg/kg orally four times daily on days -8 to -5 and cyclophosphamide 60 mg/kg iv on days -4 and -3 (Bu/Cy regimen), whereas patients with large cell anaplastic lymphoma (LCAL) and HD received BCNU 300 mg/m² iv on day -7, etoposide 100 mg/m² twice daily iv, cytarabine 100 mg/m² twice daily iv, and cyclophosphamide 45 mg/kg iv on days -6 to -3 (BEAC regimen). Children with HD received local chest irradiation in addition to BEAC.

No further chemotherapy was administered after BMT, except in one girl with CNS relapse who received cytarabine intrathecally every sixth week for 2 years. One boy with CNS relapse was also scheduled to receive cytarabine intrathecally, but treatment was discontinued due to severe headaches.
Prophylaxis and treatment of early and late effects

Infections: Antimicrobial prophylaxis consisted of trimethoprim-sulfamethoxazole and acyclovir for 3-6 months after BMT. Cataracts: cataract surgery consisted of extracapsular cataract extraction with intraocular lens implantation. YAG-laser capsulotomy was performed in patients with secondary cataract. Hypothyroidism: in cases of overt hypothyroidism, thyroxine was given in sufficient dosage to return the TSH level to the normal range and maintain T4 in the upper normal range. In cases of compensated hypothyroidism, spontaneous recovery may be awaited if the increase is borderline. We believe that thyroxine treatment should be liberal since even a marginal deficiency might contribute to impaired growth. Also, thyroid carcinoma is one of the most common secondary cancers after BMT and the possible carcinogenic effect of prolonged TSH stimulation after irradiation should be considered and may favor early treatment. After final height has been reached, thyroxine treatment should be discontinued and reassessed. Growth failure: the decision to treat with GH was based on clinical, auxiological, and biochemical data. The dosing of GH corresponded to 0.033 mg/kg body weight/day (0.1 IU/kg body weight/day). Hypogonadism and infertility; in both sexes gonadal steroid therapy should be approached with caution since epiphyseal fusion may be accelerated, resulting in a decreased height potential. This must be weighed against the risk of a low peak bone mass and future osteoporosis and the psychological impact of late pubertal development. In girls, puberty was induced using increasing doses of ethinylestradiol (starting dose 0.1 to 0.15 µg/kg/day). In boys, intramuscular injections of testosterone were mainly used (starting dose 25 mg every 4 weeks which are gradually increased until the adult dose of 250 mg every 3 to 4 weeks was reached). Liver impairment: preventive measures against hepatitis C virus included blood donor screening which was introduced in Uppsala in 1990. Renal impairment: Elevated blood pressure was treated with an ACE inhibitor. Pulmonary impairment: Idiopathic pneumonia syndrome was treated with corticosteroids.
Results and Discussion

Cataracts after autologous BMT (paper I)

Introduction and rationale for the study
Ionizing irradiation and corticosteroid treatment are the two major causes of cataracts in cancer survivors and produce morphologically the same type of cataract, so-called posterior subcapsular cataract (PSC). [39, 40] PSC is amenable to surgical correction with few complications but secondary cataract may subsequently develop. Only few studies on the long-term development of PSC and the results of cataract surgery in children have been published previously. [41-44] In theory, autologous BMT should produce fewer cataracts since large doses of corticosteroids, administered mainly to alleviate the symptoms of acute and chronic GVHD after allogeneic BMT, are seldom needed. [45, 46]

Risk factors for cataracts after the primary treatment
BMT recipients with leukemia have often previously been exposed to cranial irradiation. Although the eyes are shielded during cranial irradiation to protect the lenses, the doses used, usually ranging from 18 Gy to 24 Gy, may contribute to damaging the lens. [47, 48]

Risk factors for cataracts after autologous BMT
TBI is the major risk factor for the development of cataracts in BMT recipients. [41, 43, 44, 46, 49-51] TBI parameters reported to play a role are total dose, fractionation scheme, and dose rate. [46, 49-51] Chemotherapeutic agents given in the conditioning regimen prior to BMT, such as busulfan, may also be important. [44]

Additional risk factors for cataracts after allogeneic BMT
Corticosteroids are given chiefly after allogeneic BMT to treat and alleviate GVHD. Many investigators have reported that post-transplant corticosteroids may increase the incidence and the degree of cataracts after BMT. [45, 49-51]
Patients and methods

We describe the development of cataracts in 29 patients who were followed for at least 4 years after BMT. Median follow-up time was 8 years. Twenty-one patients were given TBI in their conditioning regimen before BMT (+TBI group) and eight patients were conditioned without TBI (-TBI group). The patients were examined prior to BMT and then annually by an ophthalmologist. One girl in the + TBI group had bilateral atrophy of the optic nerves after CNS leukemia and was excluded from tabulation of visual acuity. The indication for surgery was visual impairment affecting daily life caused by cataract.

Definitions

Best corrected visual acuity < 0.8 caused by cataract in either eye was termed significant cataract.

Results and Discussion

Cataracts before BMT

Two patients had cataracts with normal visual acuity at the time of transplant, both of whom had received cranial irradiation in their primary treatment.

Cataracts after BMT

We found that 22 out of 29 patients who had undergone autologous BMT developed cataracts within the time-frame of the study. The cataracts were always of the PSC type and, except for one child, bilateral in all cases. The median time for the first observation of PSC was 3 years and all cataracts had developed within 4 years after BMT. Except for one child with unilateral PSC, all children who developed PSC had received TBI as part of their conditioning regimen for BMT. Conversely, all those who received TBI eventually developed PSC, including one patient who received fractionated TBI. This implicates TBI as the main cataractogenic factor in this group of patients.

Previous studies have reported an incidence of PSC ranging from 70 to 100 % in patients given single fraction TBI. [41, 43, 44, 46, 49-51] In our study, the patients received 7.5 Gy in a single fraction with a midline dose rate of 0.15 Gy/min. Although our total dose of single fraction TBI is lower than that used in many centers, this is counteracted by the faster dose rate. [46] In a large series by Deeg et al it was shown that the incidence was significantly higher after TBI given in a single fraction than after fractionated TBI. [49] Subsequent studies with longer follow-up have shown that the incidence is high also after fractionated TBI, but the cataract-free
interval seems to be longer and the lens opacifications seem to be less severe, necessitating lens surgery in fewer patients. [50, 51] Traditionally the lenses are not shielded during TBI since the eyes are considered extramedullary sanctuaries. In a recent study, the effect of eye shielding during TBI was evaluated. Shielding increased the cataract-free interval and decreased the severity of cataracts compared with those not shielded while the incidence of relapse was not increased. [52]

Six of our patients (10 eyes) needed surgical repair. No cases of postoperative complications were seen. Six out of ten operated eyes needed further YAG-laser capsulotomy owing to secondary cataract. Eight patients developed significant PSC whereas twelve patients in the + TBI-group preserved visual acuity ≥ 0.8 in both eyes throughout the study. This slow progression of PSC in many patients compares favorably with previous reports after allogeneic BMT, possibly due to less need for corticosteroids after autologous BMT. [41, 43] This is supported by a recent study where the incidence of cataracts was equally high in patients treated with autologous and allogeneic BMT and TBI, but the cataract-free interval was significantly longer and the cataracts were less severe in the former group. [53]

Four patients with AML received high doses of busulfan prior to BMT, but after a median observation time of 6 years none developed PSC.

Endocrine function after autologous BMT for ALL (paper II)

Introduction and rationale for the study

BMT survivors may develop hypothyroidism, impaired pubertal development and infertility, as well as reduced final height. [36] Most previous studies of pubertal development and growth after BMT only provide short-term data. Consequently, the literature contains little information on pubertal development in its entirety and final height after BMT, and practically no data are available on final height achieved in response to growth hormone (GH) replacement therapy. [36] The possible benefit of GH therapy after BMT therefore remains unclear.

Since the primary treatment of ALL may have a large impact on the endocrine system, especially in those given irradiation, the presentation of the endocrine late effects after BMT is preceded by a short introduction to endocrine late effects after ALL.
Endocrine function after ALL

Pubertal development and fertility
Both the testis and the ovary may be damaged by cytotoxic chemotherapy (alkylating agents in particular) or irradiation, given in the primary treatment of ALL. [54] Unless the gonads are involved within the field of irradiation, Leydig cell function and ovarian function are usually sufficient to allow normal pubertal progression, however. Germ cell function is less predictable and long-term fertility may be compromised. [55, 56] Most boys given testicular irradiation after a leukemic relapse will experience complete Leydig cell failure if the dose exceeds 20-30 Gy. [57] Cranial irradiation may lead to precocious and early onset puberty, particularly in girls treated at a young age. [58]

Growth and final height
Several studies have shown a reduced final height after cranial irradiation used in the treatment of ALL. This is caused both by the combination of GH deficiency and early puberty (in girls) [59-62] Chemotherapy alone may also have an impact on final height, but different studies have produced conflicting results. [60, 63] Significant body disproportion with a relatively short spine has been reported after ALL. [58]

Growth hormone status
GH deficiency is the most common pituitary dysfunction following treatment with the comparatively low doses (18-24 Gy) of cranial irradiation used in the treatment of ALL and is probably caused by disruption of the hypothalamic control. [64, 65] The greater the radiation dose, the earlier GH deficiency will be observed. Thus, after ALL growth hormone deficiency may not become apparent for many years. [66, 67] Low dose cranial irradiation may also produce increased levels of gonadotrophins, whereas other pituitary hormones are usually unaffected. [65]

Thyroid function
ALL treatment that does not include cranial irradiation usually does not interfere with thyroid function.[68] Data on the impact of cranial irradiation are conflicting. [69, 70]

Hypothalamic-pituitary-adrenal axis
High-dose corticosteroid treatment used in the induction therapy in ALL may lead to transient suppression of the hypothalamic-pituitary-adrenal axis, which usually recovers within the first few months after treatment. [71]
BMI

In a recent study, adult survivors of childhood ALL were more likely to be overweight (BMI 25-30) or obese (BMI ≥ 30) than their adult siblings. [72] Risk factors for obesity included cranial irradiation, female sex, and young age at diagnosis.

Endocrine function after BMT

Risk factors for endocrine impairment after autologous BMT

TBI may damage both endocrine glands and the epiphyseal growth plates, resulting in impaired pubertal development, infertility as well as impaired growth. [73-78] Alkylating agents given in the preparative regimen may damage the gonads. [79] Nutritional status may have an impact on growth after BMT. [80]

Additional risk factors for endocrine impairment after allogeneic BMT

Complications occurring mainly after allogeneic BMT may cause growth impairment. These include chronic GVHD, hepatic disease, chronic diarrhea, and opportunistic infections. [79, 81, 82]

Patients and methods

We describe pubertal development and growth in 17 children (11 boys) with ALL who underwent autologous BMT which included TBI. Seven children also received cranial irradiation (CI) and five boys testicular irradiation. One girl received craniospinal irradiation and was not included in the analyses pertaining to growth. All children underwent transplantation before (n=15) or at the beginning of (n=2) puberty and reached final height. Pubertal development was assessed by the method developed by Tanner et al. [83] Height was measured with a Harpenden stadiometer. Hormonal values were measured using standard laboratory methods. For the purpose of screening, spontaneous growth hormone (GH) secretion was measured annually until final height or start of GH treatment by sampling blood every 30 minutes for 2 hours after the subjects had fallen asleep (four point GH test). In addition, children with poor growth were investigated with a 12- or 24-hour secretion curve, for which blood was sampled every 30 minutes.

Definitions

Onset of puberty was defined as a testicular volume ≥ 4 ml in boys or Tanner breast stage B2 in girls. [83] As irradiation may influence testicular volume, if other signs of pubertal development had occurred in boys while testicular volume had not reached 4 ml, puberty was considered to have begun on the basis of a combination of progression of Tanner stages and increasing levels
of testosterone. When sex steroids were given, the time of onset of puberty was defined as the start of treatment with sex steroids. [77]

Short stature was defined as height < 2 SD. Final height was considered to have been reached when the annual increase in height was less than 1 cm. GH deficiency was diagnosed, based on the result of a 12- or 24-hour secretion curve, when a peak level of GH < 24 mU/L was found during childhood and puberty and < 10 mU/L when final height had been reached. [84]

Overweight was defined as BMI 25-30 and obesity as BMI ≥ 30 in subjects who had reached final height.

Results and Discussion

**Pubertal development and fertility**

**Pubertal development in boys**

Puberty started spontaneously within the normal age range in all six boys not given testicular irradiation. Two boys were given androgen replacement therapy due to low levels of testosterone.

Most boys entered and progressed through puberty spontaneously after TBI in previous reports, although the Leydig cell may be partially damaged, as reflected by elevated LH levels but usually maintenance of normal testosterone levels. [77, 85, 86]

As expected, all boys who had received additional testicular irradiation in our study eventually developed hypergonadotrophic hypogonadism and received androgen replacement therapy. [57]

**Fertility in boys**

All but one boy had increased basal FSH concentration and all had small testicular volumes, indicating damage to the germinal epithelium and compromised fertility. [87] Semen analysis was only performed in one boy and this showed azoospermia.

Although unlikely, fertility has been described after conventional TBI in childhood. [77, 85, 86] Interestingly, in a recent study Anserini et al found that spermatogenesis recovered in 17% of adult recipients after TBI or thoracoabdominal irradiation. Recovery of spermatogenesis never occurred before the 4th year post transplant and was demonstrated as late as 9 years in one patient who was azoospermic 1 year earlier. No statistical correlation between age and recovery of spermatogenesis could be demonstrated. [88]

**Pubertal development in girls**

Puberty started spontaneously in two girls. In two girls, puberty was induced using increasing doses of ethinylestradiol, and in another two a low dose of
ethinylestradiol was given during a limited time period but was discontinued following the progression of a normal pubertal development.

Most girls undergoing BMT before the onset of puberty have a normal pubertal development, whereas those who undergo BMT after the onset of puberty may need hormonal substitution therapy. Importantly, those girls who have a normal puberty after BMT may have a shortened reproductive span and enter menopause prematurely.

The girl who received craniospinal irradiation had an early onset of puberty which was blocked with a GnRH analogue during one year. Precocious puberty is otherwise rare after BMT in girls who have been given cranial irradiation. In these girls an early onset of puberty may be counteracted by the gonadotoxicity of the conditioning regimen. [79]

**Fertility in girls**

The girl who received craniospinal irradiation became pregnant at the age of 20 years but had a spontaneous abortion after 3 months of gestation.

Even if some women are able to procreate after TBI, in two large studies the risks of preterm labor and low birthweight babies were considerably increased, probably as a result of radiation-induced changes of the uterus, whereas results pertaining to the rate of miscarriage were conflicting. [89, 90] The incidence of congenital abnormalities, developmental delay, and malignant disease in the offsprings were not higher than normal. [90]

**Growth and final height**

We found a significant decrease in height SDS at final height relative both to height SDS at BMT and to the genetic potential (target height SDS). This decrease was significant both in those who had received TBI only and in those who had been given additional cranial irradiation. The decrease occurred mostly during puberty, there being no significant loss in prepubertal height after BMT. (Fig. 1)
Figure 1 Height-SDS at BMT, at the last prepubertal visit (PUB), at final height (FH), and target height (TH). Boxes extend from the 25th to the 75th percentile. The line shows the median. Whiskers extend to the largest and smallest values within 1.5 box lengths. The dots show outlying values. Changes within groups were analyzed with the Friedman test, followed by the Wilcoxon matched-pair test. There was a significant difference between H-SDS at FH and TH-SDS (p=0.001), and a significant decrease in H-SDS between BMT and FH (p<0.001).
TBI produces growth impairment by inducing multiple endocrinological disturbances, including gonadal and thyroid dysfunction, growth hormone deficiency, and damage to the epiphyseal growth plates. [63, 73-76, 91] Of these complications, TBI-induced bone lesions may be the most important. Evidence to this effect include a severely impaired growth despite normal endocrine function and a poor growth response to GH-treatment given due to TBI-induced GH deficiency. [73, 92] TBI affects the spine in particular, which is typically reflected in a reduced sitting height to standing height ratio. [63, 73]

The reported decrease in final height in patients conditioned with single fraction TBI is about –1.5 SDS below normal, which is on a level with our results. [92, 93] In patients conditioned with chemotherapy alone, final height is usually close to normal [92, 93]

Not only the conditioning regimen but also post-transplant complications may affect growth adversely. Adan et al showed that the lack of catch-up growth after conditioning with chemotherapy alone was due to complications occurring after BMT, such as chronic GVHD, hepatic disease, chronic diarrhea, and opportunistic infections. [81] A deleterious effect of GVHD (and its treatment with corticosteroids) on short-term growth was also shown by Sanders et al. [82] In a subsequent study, Sanders et al observed catch-up growth following resolution of chronic GVHD and cessation of corticosteroid treatment in those conditioned without TBI, but not in those conditioned with TBI. [79] Cohen et al found no significant adverse effect on final height of GVHD, but a trend to more impairment of growth with increasing severity of GVHD. [93] They also included a group of children who had undergone autologous or syngeneic BMT in their study and found that the degree of height loss from BMT until final height was similar to those treated with allogeneic BMT. [93]

**Growth hormone deficiency**

We found a peak GH value of < 24 mU/L in six children when tested with a 12- or 24-hour secretion curve and in another three children when tested with the four point GH test.

GH deficiency has been reported in many studies after BMT, particularly after a combination of cranial irradiation and TBI, but also in 20 % to 70 % of subjects after TBI alone. [63, 74, 75, 82, 86] The radiation threshold required to induce GH deficiency after TBI has been suggested to be 8 Gy. [92] However, among our children given 7.5 Gy in a single fraction, three children without previous CI were found to be GH deficient.

Most studies after TBI have reported impaired GH secretion in response to provocative stimuli. [74, 75, 82, 86, 94] This may be an insensitive method to diagnose irradiation-induced GH deficiency and assessment of spontaneous GH secretion may be preferable. [65] Other biochemical
markers are often used in the diagnosis of GH deficiency, such as insulin-like growth factor 1 (IGF-1) and insulin-like growth-factor binding protein 3 (IGFBP-3) which is the major carrier of IGFs in the circulation and the most GH dependent. These markers seem to be unreliable as estimates of GH status in patients with cranial irradiation-induced GH deficiency, however, and may perhaps be even less helpful after TBI-induced GH deficiency due both to impaired synthesis and end-organ resistance (because of damage to the liver and the bones). [67, 75, 82]

In view of the diagnostic difficulties, the diagnosis of GH deficiency should be based on auxiological, clinical, and lastly on biochemical parameters: in patients in whom clinical findings indicate the possibility of GH deficiency, the finding of GH values below a predetermined cut-off level favors the diagnosis.

**Growth hormone treatment**

Ten children were treated with GH for a period of time ranging between 2 to 8 years. One patient discontinued treatment due to poor response after 2 years. In the other patients, GH treatment was continued through to final height. Six patients were re-tested with a 24-hour secretion curve after having attained final height and discontinued GH treatment. Two patients had peak GH levels < 10 mU/L. We decided not to re-institute GH so far in these patients, as well as in the four untested patients, since we judged that there was no clinical indication for treatment.

The aim of GH therapy is to achieve catch-up and, once this is done, to ensure normal growth until final height is achieved. Most studies on the effect of GH treatment after BMT have shown an increased height velocity over the first year of GH treatment as compared with the pretreatment velocity. [73-75] The increase in growth velocity is smaller than that seen in children with idiopathic GH deficiency, which is probably caused by the bone lesions induced by TBI. [73] The short-term growth response provides little help in predicting future growth and final height, however. In a multivariate analysis we found a beneficial effect of GH treatment on growth up to final height, a finding not previously reported, although Cohen et al found a trend towards better growth in their GH-treated subjects. [93]

Besides impairment of growth, the metabolic and cardiac consequences, and the impairment of quality of life caused by GH deficiency must also be considered, the improvement of which constitutes the rationale for continuing GH therapy in adulthood. Murray et al found only minor improvements in body composition, lipid profile, and bone mineral density but dramatic improvement in quality of life after 1 year of GH replacement in GH-deficient survivors of childhood cancer. [95] The incidence of clinically significant GH deficiency in adult survivors of BMT in childhood and the possible benefit of GH treatment is unknown at present.
Recently, Swerdlov et al reported an increased risk of colorectal cancer and Hodgkin’s disease in children treated with GH. [96] There is today no evidence of an increased risk of leukemic relapse or development of a second malignancy with GH therapy in ALL survivors, but data are sparse for patients who have already experienced a relapse. [97] It has therefore been recommended that, in order to avoid the time during which tumor relapse is most frequent, one should start GH no earlier than 2 years after irradiation. [98]

**Thyroid function**

One girl received thyroxine replacement before BMT and continued this treatment after BMT. Eleven children received thyroxine replacement after BMT.

Hypothyroidism is very common in children after TBI and is usually compensated (high TSH with normal thyroxine).[78, 86, 99, 100] Compensated hypothyroidism may progress to overt hypothyroidism with time but it may also recover spontaneously.[78, 99]

**Hypothalamic-pituitary-adrenal axis**

A few patients were treated with a short course of corticosteroids to relieve symptoms of post-irradiation syndrome. Only one girl received prolonged corticosteroid therapy for idiopathic pneumonia syndrome. Two patients were given cortisol replacement after BMT; one boy with borderline cortisol values and complaints of fatigue and one girl with low cortisol values shortly after BMT. Cortisol levels were normal in the other patients.

Sanders et al reported that 24% of their patients had subnormal 11-deoxycortisol levels after BMT which includes TBI. This was diagnosed by metyrapone stimulation, which may be an overly sensitive method. [82] Subsequent investigators have found normal adrenocortical function after BMT which includes TBI. [86, 100]

**BMI**

Median BMI at final height was 19 (range 16 to 30). One girl was diagnosed as overweight (BMI 26) and one boy as obese (BMI 30). This is in line with previous data that show that, in contrast to ALL, BMT survivors usually have normal or low BMI when conditioning included TBI. [101]
Hepatic function after autologous BMT (paper III)

Introduction and rationale for the study

BMT may result in impaired hepatic function both in the acute setting and in the long-term perspective. Although a number of studies on acute liver disease after BMT have been published previously, data on long-term hepatic impairment are very sparse. [102] Due to the vast geographic differences in the prevalence of viral hepatitis, an important etiologic factor for hepatic impairment in bone marrow recipients, the validity of the data that do exist may be limited. [102-104] Most previous studies have been performed in recipients of allogeneic bone marrow. Chronic hepatic impairment may be more common after allogeneic BMT than after autologous BMT due to chronic GVHD. [102-105]

Risk factors for hepatic impairment after autologous BMT

Risk factors for acute hepatic impairment (as documented by an increase in biochemical parameters) are multiple and almost inextricably interrelated. They include drug toxicity, irradiation, and infections. [105, 106] Risk factors for chronic hepatic impairment include infections and iron overload [102-104] Risk factors for so-called veno-occlusive disease (VOD, see below) include elevated transaminases before BMT, advanced stage-malignancies, and use of high-dose preparative regimens including busulfan and TBI. [107, 108]

Additional risk factors for hepatic impairment after allogeneic BMT

Acute and chronic GVHD may both damage the liver per se and predispose to hepatic infections. [102, 103, 106] Risk factors for VOD include matched unrelated donor transplantation [107, 108]

Veno-occlusive disease

Veno-occlusive disease (VOD) is the most common acute hepatic syndrome occurring after BMT and is distinguished by signs of liver impairment concurrent with fluid retention and weight gain. [107] It results from extensive damage by the conditioning regimen to structures in zone 3 of the liver acinus (centrilobular area) which may or may not include hepatic venular occlusion. Pathologically, VOD is defined as a progressive and concentric narrowing of the lumina of small intrahepatic venules associated with necrosis of hepatocytes in the centrilobular areas. Clinically, VOD is defined according either to the criteria of McDonald et al (Seattle criteria; within 30 days two of three clinical manifestations; painful hepatomegaly, weight gain, and hyperbilirubinemia) or to those of Jones et al (Baltimore criteria, which are stricter and require all three clinical manifestations within
21 days). Fluid retention with resultant weight gain is due to renal sodium retention, which in turn is related to the development of intrasinusoidal hypertension due to obstruction of hepatic blood flow. The majority of patients developing severe VOD also develop acute renal impairment and the severity of liver impairment predicts the need for hemodialysis due to acute renal impairment. [108, 111]

Patients and methods
Here, we describe hepatic function in the 40 patients who were followed up for at least 6 months after BMT. Twenty-six patients were given TBI (+TBI group) and fourteen patients were conditioned with chemotherapy alone (-TBI group). The follow-up time was a median of 10 years. Evaluation of hepatic function before and after BMT included serial measurements of alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase, bilirubin, and prothrombin time. All patients were screened for the presence of antibodies to hepatitis A virus, hepatitis B surface antigen, Epstein-Barr virus, cytomegalovirus (CMV), and toxoplasmosis. Screening for hepatitis C virus was introduced in 1990. When indicated, blood samples were also tested for HCV and CMV by the PCR technique.

Definitions
Hepatic impairment before and after BMT was defined as ALT > 1.5 μkat/L. Veno-occlusive disease of the liver was diagnosed according to the Baltimore criteria. Hepatitis due to CMV or HCV was diagnosed based on the presence of IgM, preferably confirmed by PCR, and concomitant liver impairment. We also took the appearance of a positive PCR in a previously negative patient as evidence of CMV or HCV infection, if accompanied by liver impairment.

Results and discussion

Hepatic impairment before BMT
The levels of ALT were normal in both groups before BMT and only one girl in the +TBI group had elevated ALT, but otherwise normal liver parameters. No patient was HCV positive.

Acute hepatic impairment after BMT
A large proportion of patients had transiently elevated ALT values during the early post-transplant period but only one patient in each group was diagnosed with VOD. The low incidence of VOD in our patients compares favorably with previous reports and may be explained by the lack of liver
impairment before BMT, the use of autologous as opposed to allogeneic BMT, and by the use of the stricter Baltimore VOD criteria. [108, 112-114]

**Chronic hepatic impairment after BMT**

The elevated ALT values normalized rapidly after BMT and there was no apparent difference in the evolution of ALT values between those conditioned with or without TBI (Figs. 2 and 3). During follow-up, two patients had transient elevations of ALT of unknown etiology. One girl was diagnosed with HCV infection 9 months post-transplant after a packed erythrocyte transfusion. She had slightly elevated aminotransferases at diagnosis, which then normalized. A liver biopsy performed 8 years after BMT showed normal liver parenchyma.
Figure 2 Alanine aminotransferase during the first year after BMT. (●) Patients who received TBI in their conditioning regimen before BMT. (○) Patients who did not receive TBI. Ninety-five percent confidence intervals and number of observations are given. (... ) Patients who surpassed our cut-off level of 1.5 μkat/L.
Figure 3 Long-term development of alanine aminotransferase after BMT. (●) Patients who received TBI in their conditioning regimen before BMT. (○) Patients who did not receive TBI. Ninety-five percent confidence intervals and number of observations are given. The two patients who surpassed our cut-off level of 1.5 μkat/L were presented above and are excluded here.
Due to the large regenerative capacity of the liver, acute hepatic impairment often heals completely and late impairment is usually due to the emergence of new pathogenetic factors. [102] The low incidence of chronic hepatic impairment in our autografted population is probably explained both by the low prevalence of viral hepatitis in Sweden and by the absence of GVHD. In contrast, hepatic impairment was found in more than one-half of Italian children after allogeneic BMT in the only prospective pediatric long-term follow-up previously reported. Hepatic impairment was associated with hepatitis C virus (HCV) in half of the children whereas the etiology was unknown in the others. [102] The incidence in Spanish adult bone marrow recipients was similar and was particularly associated with iron overload, chronic viral hepatitis, and chronic GVHD. [103]

Renal function after autologous BMT (paper IV)

Introduction and rationale for the study
BMT may result in impaired renal function both in the acute setting and in the long-term perspective. [115-119] Although chronic renal impairment was described at an early date in children, few studies have focused on its long-term development beyond the first few years after BMT. [120, 121] Since evidence suggests that deterioration of renal function may be expected many years after irradiation, the assessment of long-term function after BMT which includes TBI is important and should be based on accurate methods. [122, 123] Renal impairment may be more common after allogeneic BMT, particularly due to the use of cyclosporine A [121, 124, 125]

Risk factors for renal impairment after autologous BMT
Risk factors for acute renal impairment after BMT include renal impairment at the time of transplant, VOD, sepsis, and nephrotoxic medication. [108, 111, 115, 124] TBI is probably the most important risk factor for the development of chronic renal impairment. [117-119, 126-129] Most chemotherapeutic agents used in preparation for BMT seem to have little intrinsic nephrotoxicity. [121, 130] After autologous BMT, infusion of the stored marrow may cause renal damage. [105]

Additional risk factors for renal impairment after allogeneic BMT
Acute GVHD does not appear to affect renal function adversely per se but may predispose to infections necessitating nephrotoxic antibiotics. [115, 116, 124] Chronic GVHD was a risk factor for chronic renal impairment in one study but in a large series of patients with chronic GVHD no alterations in
renal function were evident. [21, 131] Cyclosporine A may affect both early and late renal function. [121, 124, 125]

Patients and methods
We describe renal function in 40 patients who were followed up for at least 6 months after BMT. Twenty-six patients were given TBI (+TBI group) and fourteen patients were conditioned with chemotherapy alone (-TBI group). Median follow-up was 10 years. Evaluation of renal function before and after BMT included serial measurements of serum creatinine, glomerular filtration rate (GFR), effective renal plasma flow (ERPF), concentrating capacity of the kidneys, dipstick urinalysis, and blood pressure.

Definitions
Acute renal impairment was defined as at least doubling of the baseline serum creatinine during the early post-transplant period. Chronic renal impairment was defined as GFR < 70 ml/min/1.73m² occurring ≥ 6 months after BMT. Hemolytic-uremic syndrome (HUS) was defined by the triad of Coombs’ negative hemolytic anemia, thrombocytopenia, and renal impairment.

Results and discussion

Renal impairment before BMT
ERPF was higher than expected in the + TBI group before BMT and tended to be high also in the - TBI group. GFR, on the other hand, was normal in both groups, and was not increased proportionately, as could be expected. One possible explanation of these findings is that the kidneys may have been damaged during the primary treatment and that ERPF was compensatorily increased to keep the glomerular filtration within the normal range. Extrarenal factors, such as anemia, bringing about a hyperkinetic circulation, may also have been involved. In contrast, in a study by Berg et al, both ERPF and GFR were decreased before BMT [120]

Acute renal impairment after BMT
One patient in the + TBI group was diagnosed with acute renal impairment. This low incidence of renal impairment compares favorably with previous reports and may be explained by the lack of renal impairment before BMT, the rarity of VOD, and the fact that all patients were autografted, which meant an absence of GVHD prophylaxis and probably fewer infections necessitating nephrotoxic antibiotics, such as amphotericin B for suspected fungal infections. [115, 116, 121, 132]
Acute renal impairment after BMT is usually defined as at least a doubling of the baseline serum creatinine within the first few weeks to months after BMT. In two pediatric groups that had undergone (mainly) allogeneic BMT, one-half to one-third of the patients developed acute renal impairment.\([116, 121]\) The incidence is similar in adult series, but children appear to fare better and no child needed hemodialysis for acute renal impairment in the cited studies, whereas one-fourth required hemodialysis in the adult BMT-recipients.\([115, 116, 121, 124]\)

Acute renal impairment after BMT has chiefly been associated with renal impairment before BMT and with early treatment-related toxicities that lead to severe hemodynamic disturbances of the kidney, notably VOD of the liver, mimicking the hepatorenal syndrome.\([108, 111, 115, 124]\) Patients with the hepatorenal syndrome have systemic vasodilatation and a reduced mean arterial pressure, leading to a decreased renal perfusion and prerenal azotemia.\([133]\) This makes them particularly sensitive to any further decrease in renal perfusion, such as that associated with septic shock (hypotension), and with the use of medication that may induce renal vasoconstriction (amphotericin B, and possibly cyclosporin A).\([111, 115, 121, 124, 125, 134]\).

Other acute renal syndromes that have been described after BMT include the tumor lysis syndrome and, after autologous BMT, stored marrow-infusion-associated toxicity.\([111]\) The harvested marrow is stored at \(-196^\circ\text{C}\) with 10 % DMSO added as a cryoprotectant. Although progenitor cells are well maintained during freezing, storage, and thawing, granulocytes and red blood cells may be disrupted. During marrow infusion, patients are exposed to toxic cell lysis products which may produce hemoglobinuria and renal damage. In addition, DMSO may induce in-vivo red cell lysis and hemoglobinuria per se.\([105]\)

Surprisingly, acute tubular necrosis seems uncommon after BMT.\([111, 115]\)

**Chronic renal impairment after BMT**

ERPF and GFR decreased rapidly during the first 6 months after BMT. The decrease in GFR was significant only in the +TBI group and was already apparent 3 months after BMT, as reflected by a significant increase in serum creatinine (Fig. 4). GFR then stabilized, albeit at a lower level than normal (Fig. 5). Distal tubular function, as assessed by the concentrating capacity of the kidneys, remained unaffected throughout the study.
Figure 4 Serum creatinine after BMT. (●) Patients who received TBI in their conditioning regimen before BMT. (○) Patients who did not receive TBI. Ninety-five percent confidence intervals and number of observations are given. Repeated measures ANOVA was used to compare pre-transplant values of serum creatinine with those obtained 3 and 6 months after BMT. The Bonferroni procedure was used for adjusting for multiple comparisons. The values obtained 3 and 6 months after BMT in the + TBI group were significantly higher than the pre-transplant value (p<0.01), but there was no significant difference between the values obtained 3 and 6 months after BMT.
Figure 5 Glomerular filtration rate after BMT in children. (●) Patients who received TBI in their conditioning regimen before BMT. (○) Patients who did not receive TBI. Ninety-five percent confidence intervals and number of observations are given. The paired t-test was used to compare pre-transplant values with those obtained 6 months after BMT. The mean GFR decreased significantly 6 months after BMT in the + TBI group (p<0.001).
In an individual analysis of our long-term survivors, 7 of our 40 patients were given a diagnosis of chronic renal impairment. Renal function remained stable at a decreased level in all of these patients, with the exception of one boy who experienced azotemia and hypertension nine years after BMT and received treatment with enalapril. A needle biopsy sample taken from his left kidney one year after BMT showed mesangial hyperplasia and separation of endothelial cells from the basement membranes in the glomeruli.

Interestingly, the incidence of chronic renal impairment in our study is similar to that of the two pediatric studies cited above, where a significant proportion of patients experienced acute renal impairment. This suggests that the degree of late impairment is unrelated to the degree of early impairment. [116, 121]

TBI appeared to be the most important risk factor for development of chronic renal impairment in our study, i.e., all patients who developed chronic renal impairment had received TBI in their conditioning and, as mentioned above, the decline in glomerular function was significant only in the irradiated group. Other findings that support the notion of TBI-induced renal damage are the clinical course and the renal biopsy changes described above, which are similar to observations described for radiation nephritis. [119, 122]

Some of the first and best descriptions of late renal impairment after BMT derive from pediatric populations. [117-119] Guinan et al described renal impairment in a series of children after BMT for relapsed ALL or stage IV neuroblastoma. [118] One-third of their long-term survivors presented with evidence of renal disease a median of 5 months after BMT. Features of renal disease included azotemia and concurrent anemia, evidence of intravascular hemolysis, thrombocytopenia, hypertension, and microscopic hematuria. Antignac et al described the histopathologic changes in seven children with late renal impairment. [119] Biopsies showed prominent glomerular changes with extensive mesangiolysis, mesangial proliferation, and focal thickening and splitting of the glomerular basement membranes. These clinical and pathological presentations are consistent with acute radiation nephritis as originally described by Luxton et al. [122]

In order to elucidate the nephrotoxic role of TBI in detail, investigators in Milwaukee have defined a renal syndrome termed BMT Np, which is characterized by increased serum creatinine, decreased GFR, hypertension, and anemia occurring more than 100 days after TBI in the absence of other identifiable nephrotoxins. [126-129] BMT Np typically presents 6-12 months after BMT, a time period which is probably related to the slow rate of replication of endothelial cells (every 2-3 months) and the cumulative incidence after 1 and 2 years is about 20% to 25%. The evolution of BMT Np is usually biphasic with an initially rapid loss of renal function (GFR), followed by a slower phase or even long-term stabilization.
Two different clinical patterns of BMT Np have been distinguished; an ‘acute’, HUS-like form (with thrombocytopenia and evidence of hemolysis) with a rapid decline in renal function and a ‘chronic’ form (without apparent hemolysis) with a slower decline and ultimately stabilization, albeit not recovery, of renal function.[127] HUS has repeatedly been described after BMT and is part of a larger syndrome characterized by microthrombi in arterioles and capillaries. [135, 136] The similarities between BMT Np and HUS are striking and perceived by some investigators to represent parts of a continuum of the same process that involves damage to the renal endothelium [118, 135-137] None of our patients was diagnosed with HUS, and GFR remained stable throughout the study. The clinical course of our patients thus seems to conform to the ‘chronic’ form described above.

Intriguingly, Luxton et al identified the renal tolerance dose as being about 20 Gy in their original description of radiation nephropathy (defined as the maximum dose that can be given to normal tissues in the irradiated volume without exceeding a 5 % incidence of serious complications in the first 5 years after irradiation) - a considerably higher dose than that used in conjunction with BMT. [122] One explanation of this discrepancy may be that Luxton used smaller smaller fractions and longer interfractionation intervals (1.5-2 Gy daily vs 1.5 Gy three times daily during 3 days employed by the Milwaukee group), allowing radiation damage to repair between fractions. Another explanation might be that today's intensive chemotherapy regimens may potentiate radiation, as has been shown for some cytostatic agents, such as busulfan, cisplatinum, and BCNU in experimental BMT Np. [138, 139] Other factors, such as nephrotoxic antibiotics, may also be important in this regard, although no antibiotics have been shown to potentiate radiation in experimental BMT Np. [139] The only apparent difference in the + TBI group in our study between the patients who by definition developed chronic renal impairment and those who did not, was that the former group had received more nephrotoxic antibiotics (a combination of vancomycin and aminoglycoside) during the early post-transplant period (16 vs 71%, Fisher’s exact test: p=0.014). Vancomycin and aminoglycoside are known to be more nephrotoxic when given in combination than when given separately [140] Here, this combination may have potentiated the effect of radiation on long-term glomerular function (Fig. 6).
Figure 6 The impact of nephrotoxic antibiotics (aminoglycoside, vancomycin, and the combination of both) on post-transplant GFR in the + TBI group. One-way ANOVA was used to compare the means of the groups. The Bonferroni procedure was used for adjusting for multiple comparisons. Excluding those who had received only vancomycin (n=3), the only significant difference was that between those who had received no nephrotoxic antibiotics and those who had received both aminoglycoside and vancomycin (p=0.007).
The hypertension seen in BMT Np should be treated vigorously. Experimental evidence suggests that antihypertensive treatment with ACE inhibitors and angiotensin II receptor blockers may not only control hypertension in BMT Np patients but may also check deterioration in renal function. [129].

Pulmonary function after autologous BMT (paper V)

Introduction and rationale for the study
Pulmonary complications are a major cause of morbidity and mortality in the early period after BMT, both in children and adults. [141, 142] Long-term pulmonary sequelae of BMT are well-recognized in adults, but less is known about the long-term effects of this procedure on lung function in children. Since pediatric lung function has to be assessed in the context of continuous growth, children and adults should be studied separately. Most previous studies have been performed in patients treated with allogeneic BMT. [143-145] The current follow-up is an extension of previous work by Arvidson et al. [146]

Risk factors for pulmonary impairment after autologous BMT
TBI is probably the most important risk factor for the development of chronic pulmonary impairment, resulting in both restrictive impairment and decreased gas exchange. [143, 144, 147, 148] Methotrexate, cyclophosphamide, busulfan, and BCNU used in the conditioning regimen may cause pulmonary impairment [149]

Additional risk factors for pulmonary impairment after allogeneic BMT
GVHD may damage the lung per se and predispose for both early and late infections, most importantly for cytomegalovirus infection.[141, 142, 150, 151] Of late non-infectious pulmonary complications, particularly obstructive impairment has been associated with chronic GVHD.[152]

Idiopathic pneumonia syndrome
Of noninfectious complications, idiopathic pneumonia syndrome (IPS) is the most important and carries a high mortality rate. It occurs 1.5-6 months after BMT and is defined as diffuse lung damage for which an infectious etiology is not identified. [153, 154] It is less common after autologous than after allogeneic BMT. [155]
Patients and Methods

We describe pulmonary function in 26 children who were followed up for at least 60 months after BMT with repeated pulmonary function tests (PFTs) and who had received no radiation to the thorax other than TBI. Twenty patients were given TBI (+TBI group) and six patients (-TBI group) were conditioned with chemotherapy alone. Median follow-up was 10 years. PFTs included measurement of lung volumes, of dynamic spirometry, and of the diffusing capacity of the lung for carbon monoxide (DLCO). To make lung function variables comparable, they were expressed as a percentage of predicted values in each subject.

Definitions

Restrictive lung disease was defined as TLC < 80% of predicted, obstructive lung disease as FEV1/VC < 70, and isolated diffusing impairment as DLCO < 80% of predicted with TLC in the normal range. The diagnosis of idiopathic pneumonia syndrome (IPS) was made in patients who developed pulmonary symptoms and findings indicative of IPS on radiographs but in whom no etiological infectious organism could be verified despite extensive examinations, including bronchoalveolar lavage. [153] Pulmonary complications after BMT were classified as late if occurring >100 days after BMT.

Results and discussion

Pulmonary impairment before BMT

We found restrictive impairment in one of nineteen tested patients and isolated diffusing impairment in four of nine tested patients, all of whom had received treatment for ALL. Fanfulla et al reported that lung function abnormalities were common before BMT in children treated for hematological malignancies, with a trend to more impaired baseline lung function in patients who had been treated for a longer time with chemotherapy before BMT. [143]

Early pulmonary complications

Early pulmonary complications of clinical significance were noted in three patients; pulmonary edema syndrome in one boy 10 days after BMT, pneumonia, probably caused by Candida albicans, in one boy 14 days after BMT, and idiopathic pneumonia syndrome (IPS) in one girl 6 weeks after BMT. All improved rapidly after institution of therapy. This low incidence is probably related to the absence of GVHD and the sustained host immunity after autologous BMT.
Impaired pulmonary function tests
In addition to clinical syndromes after BMT many patients develop impaired lung function as assessed by PFTs, which is often subclinical. Patterns of restrictive impairment and decreased gas exchange predominate after both autologous and allogenic BMT. [143-145]

Restrictive impairment
The mean lung volumes (TLC and VC) in the + TBI group decreased rapidly during the first 6 months after BMT and then recovered (VC proportionately more), but were still modestly decreased 5 years after BMT at 10 % below baseline (Fig.7). The proportions of children in the + TBI group with restrictive impairment 5 and 10 years after BMT were 4/20 (20%) and 3/14 (21%). No child in the – TBI group had persistent restrictive impairment. In a multivariate analysis including 15 patients who had received TBI and four patients who were conditioned with chemotherapy alone, TBI was shown to have a significant impact on the decrease in TLC after BMT. Although these results show that TBI decreases TLC, due to the small sample size (especially the – TBI group), the conclusion that TBI causes restrictive lung disease may not be supported.
Figure 7 Total lung capacity values in children who received TBI in their conditioning regimen before autologous BMT. Values are expressed as percentages of predicted. Ninety-five percent confidence intervals and number of observations are given. The paired t-test was used to compare pre-transplant values with those obtained 5 years after BMT. The mean TLC obtained 60 months after BMT was significantly lower than the pre-transplant value (p<0.01).
TBI has previously been associated with decreased lung volumes in adults, but data for children are conflicting. [143, 144, 147, 148] One reason for this may be the small sample sizes in pediatric studies and the heterogeneity with respect to TBI regimen. Single-fraction TBI has been associated with a more marked decrease in lung volumes and less recovery in comparison with fractionated TBI. [148] We found no benefit of fractionation in our small sample, however. On the contrary, those who had received fractionated TBI had a larger decrease in TLC than those who received single fraction TBI. A possible sparing effect of fractionation may have been counteracted by the 60% higher total dose in those who received fractionated TBI, but our limited data should be interpreted with caution.

Obstructive impairment
FEV₁ followed a biphasic pattern roughly similar to that of the lung volumes. Since FEV₁ changed in parallel with the lung volumes, the mean FEV₁/VC remained unchanged through the follow-up period. Only one patient was diagnosed with obstructive impairment. Investigators generally report a proportionately lower incidence of obstructive compared with restrictive changes in children after BMT. [143-145] In addition, the low incidence in our study may partly be due to the absence of chronic GVHD in our group of autografted children. [152]

Isolated diffusing impairment
The mean DLCO in the + TBI group recovered from a nadir 6 months after BMT and was not significantly different 5 years after BMT from the baseline value. (Fig. 8) The proportions of children in the + TBI group with isolated diffusing impairment at 5 and 10 years were 7/20 (35%) and 7/13 (54%), respectively. The children who had received chemotherapy only in their conditioning regimen showed isolated diffusing impairment as the only long-term sequela in 4/5 and 1/3 at 5 and 10 years, respectively.

Impaired gas exchange after BMT has been associated with TBI in previous studies. [147, 148] Our data suggest that apart from TBI, chemotherapy may have a pathogenetic role in the induction of impaired gas exchange. This finding is supported by results of other investigators. [143, 144, 156] Of the chemotherapeutic agents used in our patients both before and in conjunction with BMT, methotrexate, cyclophosphamid, busulfan, and BCNU have been linked with the development of pulmonary fibrosis which might lead to thickening of the pulmonary diffusion membrane. [149]
Figure 8 The diffusion capacity for carbon monoxide in children who received TBI in their conditioning regimen before autologous BMT. Values are expressed as percentages of predicted. Ninety-five percent confidence intervals and number of observations are given. The paired t-test was used to compare pre-transplant values with those obtained 5 years after BMT.
**Respiratory symptoms**
Like most long-term studies there was a conspicuous lack of spontaneously reported respiratory symptoms in our patients. These data were based on a retrospective chart review, however, and less severe symptoms may have been missed. On the other hand, we did not measure exercise capacity but only lung function at rest. In one study, one-fourth of patients with restrictive impairment after BMT had exertional dyspnea. [157]

**Body proportions**
We found a relationship between height SDS and TLC suggesting a clinically significant body disproportion, which may result in erroneous estimations when children given TBI are compared with a reference population. [158] Since body disproportion with a relatively short spine has been reported to occur frequently after ALL and BMT this should always be considered. [58, 63, 73] The median sitting height to standing height ratio being normal in our group of patients, we do not believe that the influence of body proportion mattered, at least on the group level.
Conclusions and clinical implications

Paper I

The incidence of posterior subcapsular cataract (PSC) was high after autologous BMT where the conditioning regimen included TBI. Although all patients who received TBI developed PSC within 4 years after BMT, the visual acuity was well preserved in many patients throughout the study, reflecting a slow progression of PSC. This may be due to the lack of corticosteroids after autologous BMT.

Visual development is not completed until around 8 to 10 years of age. The amblyogenic role of cataract in children is therefore important. The cataract itself may cause visual impairment which may be superimposed by amblyopia in the incompletely developed eyes of a child. Since PSC is a side-effect with significant implications for the child that is amenable to a treatment with few complications, it is imperative that it should not be missed. Regular ophthalmologic investigations are therefore necessary, especially in patients who have received TBI and in the small children who may not complain of a slowly progressing visual impairment. This reasoning also applies after lens surgery, due to the risk of secondary cataract.

Paper II

The impairment of pubertal development and the loss of final height were in line with reports after allogeneic BMT.

Although preliminary, our results suggest a beneficial effect of growth hormone (GH) treatment and we believe it to be a reasonable policy to administer GH after BMT to those children with a decreased growth rate and low GH values, presuming that other hormones necessary for optimal growth are adequately replaced. Until reaching final height, all children should be regularly monitored regarding growth velocity and pubertal development at least every six months. Clinical suspicions of GH deficiency should be confirmed by measuring GH secretion during 24 hours. All subjects receiving GH treatment should be entered into central registries to allow surveillance and monitoring of the efficacy and safety of GH therapy.
Paper III

After the immediate post-transplant period hepatic function was well preserved over a period of 10 years after autologous BMT. TBI did not appear to be a risk factor for the development of chronic hepatic impairment.

Paper IV

Renal function as assessed by the renal plasma flow may be impaired to a larger extent after the primary therapy than what is obvious when only the glomerular filtration rate is measured.

After the first year there was little change at all over a period of 10 years after autologous BMT and the main impression was that renal function was comparatively well preserved in the long-term. TBI appeared to be the most important risk factor for development of chronic renal impairment. Nephrotoxic antibiotics may have contributed to the renal impairment and should be avoided if possible.

Persistent hematuria and/or proteinuria and late hypertension in one patient indicate ongoing renal disease. This, together with experimental data indicating that further deterioration can be anticipated more than ten years after TBI, emphasizes the need for life-long regular follow-up in these patients. Since long-term survivors after BMT have a long life expectancy, also the age-related deterioration in renal function may be important. All patients who have received TBI should be regularly evaluated at least with blood pressure and serum creatinine and antihypertensive treatment should be initiated in hypertensive patients.

Paper V

The same pattern of restrictive pulmonary disease and impaired gas exchange as we have described has been reported by others and is compatible with an acute injury with subsequent, though often incomplete, repair.

After the first year there was little change at all over a period of 10 years after autologous BMT and the main impression was that lung function was comparatively well preserved in the long-term. TBI appeared to be the most important risk factor for development of restrictive pulmonary disease, whereas chemotherapy also might have been of importance for development of impaired gas exchange.

Although pulmonary function was decreased in a number of patients 10 years after BMT, it was not clinically overt in any of them. In some, the age-related deterioration in pulmonary function may entail progression to
clinically overt disease. BMT survivors must therefore avoid any factor that may quicken this deterioration and must, in particular, be strongly recommended to avoid smoking.

Yearly tests of pulmonary function are not indicated in patients free of symptoms. However, there is a need for continuing follow-up, preferably including tests performed during exercise, with 5- to 10-year intervals.
Future aspects

Our results emphasize the detrimental role of irradiation on organ function after BMT. The dwindling use of cranial irradiation in ALL patients and the introduction of fractionated TBI in the conditioning regimen prior to BMT probably will result in less severe sequelae. Shielding of the eyes and of the kidneys during TBI, as well as of other organs, may be options for the future. Studies are currently underway which explore additional methods to prevent the development of post-irradiation complications, such as captopril, an ACE-inhibitor, given prophylactically to prevent BMT nephropathy.

Hitherto, the growth impairment observed after irradiation has taken precedence when deciding whom to treat with growth hormone. Considerations pertaining to the metabolic profile and the quality of life will probably be increasingly important in the future.

New techniques to circumvent infertility have been developed and are successively being introduced into the clinical practice.

Problems of long-term follow-up

Since our follow-up program was launched in the eighties, a number of studies from our and other centers have helped to clarify the nature of late effects after BMT and have forwarded recommendations for the design of future follow-up programs. As outlined above, important therapeutic changes are successively being introduced into the clinical practice which may render these recommendations obsolete. This illustrates the basic dilemma of long-term follow-up, that current follow-up programs are often based on outdated therapies. They must therefore be continuously up-dated to conform with the present clinical reality.

As some late effects develop only after many years or become progressively severe with time there is often a need for very long follow-up. This poses a number of questions, such as which investigations should be included in the follow-up program, where the follow-up should be performed, by whom, and for how long. Should long-term survivors be followed at dedicated follow-up units where follow-up research also is conducted, or should they be followed within the existing health care system where knowledge of their therapeutic exposures often is minimal? How
should the transition to adult health care be conducted? What is the psychological burden of regular long-term follow-up in patients cured of the original disease but continually treated as being ‘at risk’? To deal with these and related questions, a Swedish late effects working group (SALUB) was constituted 2 years ago and a Nordic group was recently constituted in Umeå in May 2003 as the latest member of the NOPHO family.
Acknowledgements

I wish to express my sincere gratitude to:

**Gudmar Lönnerholm** – my main tutor, for introducing me to the field of clinical research, for his enthusiasm, patience and support, as well as for his vast knowledge of and participation in all aspects of the research process, from the planning and scrupulous maintenance of the follow-up program to the intuitive editing of the final manuscripts.

**Jan Gustafsson** – my co-tutor, for his never-failing enthusiasm, for his swift and accurate reading of manuscripts, for always being available for questions whether being in his room at the hospital or out sailing in the Stockholm archipelago, and for sharing with me his vast knowledge in pediatric endocrinology.

**Torsten Tuvemo** – for providing excellent conditions for clinical research.

**Johan Arvidson** – co-author, former room-mate, and forerunner, who left behind him a trail that has made much easier the trek through the forbidding jungle of causes and effects after BMT, for in-depth reading of manuscripts, and for being largely responsible for my choice of clinical speciality.

**Lars-Erik Bratteby** and **Hans Hedenström** – co-authors, for sharing with me their special knowledge in clinical physiology and for fruitful discussions.

**Hans Hagberg, Agneta Mandahl, Per Söderberg, Gunnar Öberg, and Kristina Carlson** – co-authors, for sharing with me their special knowledge. Statisticians at Statisticon for expert statistical advice.

**Steve Scott-Robson, Maud Marsden, and Nigel Rollison** for expert linguistic advice.

Colleagues and staff at the Children’s Hospital, especially **Britt-Marie Frost** for last-minute help with the redoubtable mall, among many things.

**Inga Andersson** and **Barbro Westerberg** – for assistance with practical matters.

Family and friends.

This study was supported by the Children’s Cancer Foundation in Sweden.
Summary in swedish

Bakgrund


Patienter med hematologiska sjukdomar behandlas inför autolog BMT med cytotoxika till komplett remission (ingen kvarvarande detekterbar sjukdom), varpå den egna märgen tas ut och frysas ned, eventuellt efter förbehandling. Letala doser cytotoxika ges därefter, vid vissa diagnoser kombinerad med helkroppsbestrålning. Behandlingen är mycket benmärgstoxisk, och den utradade blodbildande förmågan ‘återskapas’ med infusion av den tidigare skördade benmärgen.

Långtidsbiverkningarna efter autolog BMT är endast ofullständigt kända. Procedurmortatalen vid autolog BMT är låg. All immunosuppressiv behandling avslutas i anslutning till transplantationen och risk för så kallad graft-versus-host disease (GVHD), där givarceller angriper mottagaren efter alllogen BMT, finns ej. Långsiktiga biverkningar av strålning och cytotoxika var för sig finns utförligt beskrivna. Vid autolog BMT prövas dock kombinationer som man tidigare har relativt ringa erfarenhet av. Om det rör sig om ett återfall i sjukdomen har patienten dessutom en tidigare behandlingsomgång bakom sig, inkluderande kemoterapi och i förekommande fall bestrålning av hjärna, testiklar, hals, thorax, och buk.
Sedan 1985 har 50 barn genomgått autolog BMT i Uppsala, främst på grund av återfall i akut lymfatisk leukemi. Andra indikationer har varit högriskleukemi, akut myeloisk leukemi, recidiverande Hodgkins sjukdom och recidiverande non-Hodgkin-lymfom. För att kunna beskriva eventuella biverkningar har samtliga patienter ingått i en omfattande prospektiv långtidsuppföljning i minst 10 år. Samtliga har följts upp före BMT, var tredje månad under det första året efter BMT, och därefter årligen.

En noggrann utvärdering av biverkningarna efter autolog BMT på barn har stort värde för att klarlägga behandlingsmetodens egenskaper, och kan också indirekt belysa biverkningarna efter allogen BMT.

**Delarbete I**

**Bakgrund:** Att helkroppsbestrålning ger upphov till katarakt är väl känt. Endast ett litet antal studier har dock studerat kataraktutvecklingen hos barn, och då efter allogen BMT. Barn som genomgått allogen BMT har ofta fått behandling med kortikosteroider för att lindra GVHD. Kortikosteroider kan ge upphov till katarakt i sig, alternativt förvärva strålningsinducerad katarakt.

**Patienter och metoder:** Tjugonio patienter med recidivfri överlevnad mer än 4 år efter BMT inkluderades i studien. Tjugoen patienter fick helkroppsbestrålning i samband med BMT. Alla patienter följdes upp med årliga kontroller av ögonläkare. **Huvudsakliga resultat:** Två patienter hade katarakt före BMT. Samtliga barn som fått helkroppsbestrålning utvecklade katarakt inom 4 år medan endast ett barn som inte fått helkroppsbestrålning utvecklade katarakt. Kataraktutvecklingen var förhållandevis långsam. Under uppföljningen (median 8 år) behövde sex patienter genomgå kataraktoperation. **Slutsats:** Liksom efter allogen BMT fick samtliga katarakt som fått helkroppsbestrålning (givet i en seans). Förloppet var möjlichen långsammare jämfört med allogen BMT.

**Delarbete II**

**Bakgrund:** Att cytostatika och helkroppsbestrålning i samband med BMT ger upphov till skador på det endokrina systemet är väl känt. Detta leder till hormonella störningar som påverkar både pubertet och långdöttillväxt. Dessutom ger helkroppsbestrålning upphov till skelettskador som ytterligare minskar långdöttillväxten. Ett flertal kortidsstudier av pubertetsutveckling och tillväxt efter BMT har genomförts tidigare, framför allt efter allogen BMT. Barn som genomgått allogen BMT får i förekommande fall långvarig behandling med kortikosteroider för att lindra GVHD vilket kan ge upphov till ökande tillväxthämnings. Mycket få studier har följt barnen ända till slutlängd. En fråga som är viktig att besvara är huruvida
tillväxthormonbehandling av barn med tillväxthormonbrist kan öka tillväxten efter BMT trots de strålningsinducerade skelettskadorna. **Patienter och metoder:** Sjutton patienter (elva pojkar) som behandlats för akut lymfatisk leukemi inklusive BMT före pubertetsstart och som nått slutlängd inkluderades i studien. Samtliga patienter fick helkroppsbestrålning i samband med BMT. Fem pojkar hade dessutom tidigare fått strålbehandling mot testiklarna och sju barn mot hjärnan (kraniell bestrålning). Alla patienter följes upp med klinisk kontroll för bedömning av pubertetsstadium och längd, könshormonbestämning samt bestämning av tillväxthormon. **Huvudsakliga resultat:** Samtliga pojkar som inte fått extra testikelbestrålning startade en normal pubertet men fick understödjas hos två pojkar med manligt könshormon på grund av låga hormonvärden. Samtliga pojkar som fått extra testikelbestrålning behandlades med manligt könshormon. Två flickor behövde hjälp med att starta och understödja puberteten med kvinnligt könshormon. Slutlängden var kortare än normalt hos samtliga utom två patienter. Tio patienter fick tillväxthormonbehandling i mellan 2 till 8 år. Faktorer som påverkade tillväxten negativt var kranial bestrålning, låg ålder vid BMT samt kort duration av tillväxthormonbehandlingen. ** slutsats:** De kunde påvisa en positiv behandlingseffekt av tillväxthormon trots en förmodad strålningsinducerad skelettskada.

**Delarbete III**

**Bakgrund:** Ett flertal tidigare arbeten har studerat akuta leverbiverkningar efter BMT hos både barn och vuxna. Endast ett litet antal studier har dock studerat långtidsutvecklingen av leverfunktionen hos barn, och då efter allogen BMT. Det är dock vanskligt att extrapolera från föreliggande studier av två huvudsakliga skäl; dels kan GVHD efter allogen BMT ge upphov till leverskada i sig, dels har studierna genomförts i länder med hög prevalens av virushepatit. **Patienter och metoder:** Fyrtio patienter med recidivfri överlevnad mer än 6 månader efter BMT inkluderades i studien. Tjugosex patienter fick helkroppsbestrålning i samband med BMT. Alla patienter följes upp med klinisk kontroll samt rutinmässiga leverlaborationer. **Huvudsakliga resultat:** Levervärdena var normala före BMT. Flertalet patienter hade akut förhöjda levervärden efter BMT, men endast två patienter blev diagnosticerade med så kallad venocklusiv sjukdom, en välkänd biverkning efter kraftig cytostatikabehandling. Långtidsuppföljningen visade att de akut förhöjda levervärdena mycket snabbt normaliserades och förblev normala utan någon skillnad mellan de strålade och icke-strålade grupperna. En flicka fick hepatitis C infektion, ännu utan påtaglig leverpåverkan. ** Slutsats:** De akut förhöjda levervärdena
normaliserades snabbt och förblev normala i ett 10-årsperspektiv, vilket är gynnsamt jämfört med allogen BMT. Helkroppsbestrålning i sig verkar inte skada levern i ett långtidsperspektiv.

Delarbete IV

Bakgrund: Att framför allt helkroppsbestrålning i samband med BMT ger upphov till njurskada manifesterad 3-12 månader efter BMT har blivit alltmer uppenbart under de senaste 10 åren. Trots att experimentella data antyder att njurfunktionen kan försämras flera år efter strålningen har ingen verklig långtidsuppföljning utförts med välvaliderade metoder. Barn som genomgått allogen BMT får ofta behandling med njurtoxiska immunsupprimerande läkemedel för att förhindra utvecklingen GVHD.

Patienter och metoder: Fyrtio patienter med recidivfri överlevnad mer än 6 månader efter BMT inkluderades i studien. Tjugosex patienter fick helkroppsbestrålning i samband med BMT. Alla patienter följdes upp med klinisk kontroll, rutinmässiga njurlaborationer, mätning av renalt plasmaföd, glomerulär filtrationshastighet samt koncentrationskapacitet. Njurskada av klinisk betydelse definierades som glomerulär filtrationshastighet < 70 ml/min/1.73m² Huvudsakliga resultat: Det renala plasmaföd var högt medan den glomerulära filtrationshastigheten var normal före BMT. Sex månader efter BMT sjönk både det renala plasmafödet och den glomerulära filtrationshastigheten endast i den strålade gruppen och stabiliserades på en sänkt nivå under den fortsatta uppföljningen. Koncentrationskapaciteten var normal i båda grupper. Sju barn, samtliga strålade, hade njurskada av klinisk betydelse enligt vår definition. En ung man med njurskada klinisk betydelse fick hypertoni 9 år efter BMT. De sju som fick njurskada av klinisk betydelse hade fått signifikant mer njurtoxiska antibiotika än de övriga nitton i den strålade gruppen. Slutsats: Resultaten tyder på att helkroppsbestrålningen ger upphov till en skada som är märkbar efter 6 månader och sedan stabiliseras på en en sänkt nivå utan större förändringar i ett 10-årsperspektiv. Förutom helkroppsbestrålningen verkar njurtoxiska antibiotika spela roll.

Delarbete V

Bakgrund: Att BMT ger upphov till akut lungskada är väl känt. Endast ett litet antal studier har dock studerat långtidsutvecklingen av lungfunktionen hos barn och då efter allogen BMT. GVHD efter allogen BMT kan ge upphov till både infektiösa och icke-infektiösa lungbiverkningar. Patienter och metoder: Tjugosex patienter med recidivfri överlevnad mer än 5 år efter BMT inkluderades i studien. Tjugo patienter fick helkroppsbestrålning i
samband med BMT. Alla patienter följdes upp med klinisk kontroll, lungröntgen och lungfunktionsundersökningar bestående av mätning av lungvolym (total lungkapacitet, funktionell residualkapacitet, residualvolym, vitalkapacitet), dynamisk spirometri (vitalkapacitet, forcerad vitalkapacitet, forcerad expiratorisk volym under en sekund, forcerad expiratorisk volymprocent), samt diffusionskapacitet för kolmonoxid. Huvudsakliga resultat: Före BMT hade samtliga barn utom ett var normal lungvolym, medan fyra av nio hade sänkt diffusionskapacitet. Sex månader efter BMT sjönk lungvolym och diffusionskapacitet framför allt i den strålade gruppen, de ökade sedan något för att stabiliseras på en lätt sänkt nivå. En femtedel av barnen hade kvarstående restriktiv lungfunktionsnedsättning (klintskt betydelsefull sänkning av lungvolymen), samtliga i den den strålade gruppen, medan lika många i den icke-strålade gruppen hade kvarstående diffusionskapacitetsänkning av klinisk betydelse. Slutsats: Resultaten tyder på att helkroppsbestrålningen ger upphov till en skada som är märkbar i form av sänkt lungvolym och sänkt diffusionskapacitet efter 6 månader och sedan stabiliseras på en lätt sänkt nivå utan större förändringar i ett 10-årsperspektiv. Även cytostatika verkar kunna sänka diffusionskapaciteten.
References

13. Schmitz, N., et al., Aggressive conventional chemotherapy compared with high-dose chemotherapy with autologous haemopoietic stem-cell transplantation for
30. Ringden, O., et al., Increased risk of chronic graft-versus-host disease, obstructive bronchiolitis, and alopecia with busulfan versus total body irradiation: long-term results of a randomized trial in allogeneic marrow


A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology*. (Prior to October, 1993, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science”.)