KUOPION YLIOPISTON JULKAISUJA D. LÄÄKETIEDE 386
KUOPIO UNIVERSITY PUBLICATIONS D. MEDICAL SCIENCES 386

TARU KUITTINEN

Autologous Stem Cell Transplantation in Patients with Non-Hodgkin´s Lymphoma

Progenitor Cell Mobilisation, Toxicity of High-dose Therapy, and Progressive Disease After Transplantation

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium L22, Snellmania building, University of Kuopio, on Saturday 22nd April 2006, at 12 noon

Department of Medicine
Kuopio University Hospital and University of Kuopio

KUOPION YLIOPISTO
KUOPIO 2006

Distributor:
Kuopio University Library
P.O. Box 1627
FI-70211 KUOPIO
FINLAND
Tel. +358 17 163 430
Fax +358 17 163 410
www.uku.fi/kirjasto/julkaisutoiminta/julkmyyn.html

Series Editors:
Professor Esko Alhava, M.D., Ph.D.
Department of Surgery

Professor Raimo Sulkava, M.D., Ph.D.
Department of Public Health and General Practice

Professor Markku Tammi, M.D., Ph.D.
Department of Anatomy

Author´s address:
Department of Medicine
Kuopio University Hospital
P.O. Box 1777
FI-70211 KUOPIO
FINLAND

Supervisors:
Docent Esa Jantunen, M.D., Ph.D.
Department of Medicine
Kuopio University Hospital

Docent Juha Hartikainen, M.D., Ph.D.
Department of Medicine
Kuopio University Hospital

Docent Tapio Nousiainen, M.D., Ph.D.
Department of Medicine
Kuopio University Hospital

Reviewers:
Docent Liisa Volin, M.D., Ph.D.
Department of Medicine, Division of Haematology
Helsinki University Central Hospital

Docent Markku Kupari, M.D., Ph.D.
Department of Medicine, Division of Cardiology
Helsinki University Central Hospital

Opponent:
Professor Tapani Ruutu, M.D., Ph.D.
Department of Medicine, Division of Haematology
Helsinki University Central Hospital

ISBN 951-27-0566-4
ISBN 951-27-0583-4 (PDF)
ISSN 1235-0303

Kopijyvä
Kuopio 2006
Finland

Kuittinen, Taru. Autologous stem cell transplantation in patients with non-Hodgkin's lymphoma: progenitor cell mobilisation, toxicity of high-dose therapy, and progressive disease after transplantation. Kuopio University Publications D. Medical Sciences 386. 2006. 115 p.
ISBN 951-27-0566-4
ISBN 951-27-0583-4 (PDF)
ISSN 1235-0303

ABSTRACT

Non-Hodgkin's lymphoma (NHL) is among the leading indications for high-dose therapy (HDT) supported by autologous stem cell transplantation (ASCT). A significant proportion of NHL patients fail to mobilise a sufficient number of haematopoietic progenitor cells (HPCs) to proceed to HDT. Organ toxicity is an inevitable consequense of HDT. Cardiac toxicity is potentially the most threatening non-haematological side-effect of high-dose cyclophosphamide, which is an essential part of many HDT regimens, like BEAC. Furthermore, a large proportion of NHL patients experience progressive disease after ASCT.

The purpose of this study was to identify factors predicting HPC mobilisation in adult NHL patients. The toxicity and efficacy of BEAM and BEAC regimens were also compared. Furthermore, cardiac toxicity of BEAC was assessed by magnetic resonance imaging, radionuclide ventriculography and natriuretic peptide measurements. The factors predicting treatment response and survival after progressive disease following ASCT were identified.

Bone marrow infiltration and lower platelet count just prior to HPC mobilisation predicted moblisation failure whereas the chemotherapy score was not useful in this regard. BEAM regimen showed more toxic effects to gastrointestinal tract than BEAC. However, there was no difference in efficacy between the two regimens. BEAC regimen resulted in the very acute cardiac toxicity characterised by cardiac dilatation. Also subclinical, transient left ventricular systolic dysfunction was observed. Natriuretic peptides seem to be more sensitive than left ventricular ejection fraction in detecting this cardiac effect. Furthermore, International Prognostic Index ≤ 2, histology other than diffuse large B-cell lymphoma, longer time from ASCT to progression and normal serum lactate dehydrogenase at progression were factors predicting treatment response and survival in patients with progressive disease after ASCT.

In conclusion, important factors predicting HPC mobilisation, toxicity of HDT and prognostic factors for progressive disease after ASCT were found in this study. These observations can be used clinically to improve this treatment option in patients with NHL.

National Library of Medicine Classification: QV 269, QZ 267, WH 525, WN 185
Medical Subjects Headings: adult; antineoplastic combined chemotherapy protocols; cyclophosphamide; drug toxicity; heart/drug effects; lymphoma, non-Hodgkin/complications; lymphoma, non-Hodgkin/therapy; magnetic resonance imaging; natriuretic peptides; prognostic factors; radionuclide ventriculography; stem cell transplantation/methods; time factors; transplantation, autologous; treatment outcome

"Mikä olen? Tähdenlento Luojan ikuisessa yössä, tomujyvä aavan aineen lakkaamattomassa työssä.

Alistukaa, avaruudet, pienen tähden välkynnälle! Tahdon loistaa, tahdon laulaa, kiitoslaulun elämälle"

-L. Onerva-

ACKNOWLEDGEMENTS

This thesis was carried out in the Department of Medicine, Kuopio University Hospital in 2000-2005. First of all, I own my sincere thanks to Professor Markku Laakso, M.D., Professor Leo Niskanen, M.D. and Docent Seppo Lehto, Administrative Chief Physician of the Department on Medicine, for arranging me facilities to perform this study. They all have given me extremely wise and useful advice.

I wish to express my deepest gratitude to my principal supervisor, Docent Esa Jantunen, M.D., for suggesting this topic to me, and for leading me to the fascinating world of scientific research. I cannot find the words to thank him enough for all his help! His unfailing energy and enthusiasm have supported me during all phases of the study. I have always been impressed by his intelligence and ability to follow the "red ribbon". Even though there have been some difficult moments, his "coach-like" attitude has given me faith and strength to continue. I could tell several anecdotes about him. During the hard work we never forgot laughter and joy.

I wish to express sincere gratitude to my supervisor, Docent Juha Hartikainen, M.D., for his talented guidance and excellent advice throughout the study. I greatly admire his intelligence, experience in clinical and scientific work, and his unfailing logic. He also tried to teach me the "Essentials of Cardiology" and I really admire him for undertaking that challenge. His encouragement was crucial for me in developing the skills for scientific writing. I also appreciate that he has always been available in spite of his many commitments.

I also owe my sincere gratitude to Docent Tapio Nousiainen, M.D., my supervisor and also principal teacher in haematology. I admire his wisdom and profound knowledge both in haematology and science. I have had the privilege of working under his guidance and to become familiar with the fascinating world of haematology. His counsel has been of great importance in improving my scientific thinking.

I express my deepest gratitude to the official reviewers of this thesis, Docent Liisa Volin, M.D., and Docent Markku Kupari, M.D., for their careful and constructive help leading to the improvement of this thesis. Their skilful and encouraging criticism was one of the most valuable things during this thesis.

I am sincerely grateful to Minna Husso, Phil.Lic., and Petri Sipola M.D., PhD., Department of Radiology. I admire their logical and thorough way in doing science. I knew nothing about MRI, but they had the experience and ability to explain many difficult things in an understandable way. We had many delightful sessions, and they offered me several fruitful, ingenious comments when preparing the manuscript. It was a great pleasure to get to know you both.

I am very thankful to Eija Mahlamäki, M.D., Department of Clinical Chemistry, Professor Esko Vanninen, M.D., and Hanna Mussalo, M.D., PhD., Department of Clinical Physiology and Nuclear Medicine, Minna-Ala Kopsala, M.Sc., and Professor Olli Vuolteenaho, M.D., University of Oulu and Oulu Biocenter for their, kind and excellent collaboration and paramount comments in improving the manuscript.

I owe my sincere thanks to Pirjo Halonen, M.Sc., Information Technology Centre, University of Kuopio, for expert statistical advice in solving some difficult problems in this study. She was always ready to help with smiling face despite her busy time schedules.

I wish to thank Docent Erkki Elonen, M.D., and Riikka Räty, M.D., PhD., Helsinki University Central Hospital, Docent Tom Wiklund M.D., and Docent Sirpa Leppä, M.D.,

Helsinki University Central Hospital, Docent Kari Remes M.D., and Mervi Putkonen, M.D., Turku University Hospital, Outi Kuittinen, M.D., PhD., and Professor Taina Turpeenniemi-Hujanen, M.D., Oulu University Hospital and Docent Tuula Lehtinen, M.D., Tampere University Hospital for their excellent collaboration and clarifying comments when preparing the manuscript for this thesis.

This study would not have been possible without the excellent assistance and positive attitude of the staff in the Department of Haematology (2103). Those enjoyable moments during coffee breaks and lunch time have given me a lot of energy and good spirit to continue. I have consumed tons of coffee in preparing this thesis. I am deeply thankful to the personnel of Department of Radiology, Department of Clinical Physiology and Nuclear Medicine and Department of Clinical Chemistry for their kind help and patience: THANK YOU! Especially I want to express my sincere thanks to Helena Ollikainen, RN., whose skilful help was essential.

I am deeply indepted to Ms. Eeva Oittinen for her excellent and professional secretarial help in the processing of this thesis. She has also helped me during the years by sending six manuscripts to the "right" journals with "appropriate" covering letters. THANK YOU! I also thank Docent David Laaksonen, M.D., for the revision of the English language of my thesis, and the personel of the Scientific Library at the Kuopio University Hospital for their assintance in collecting the literature.

I wish to thank all my dear colleagues in the Department of Medicine for creating such an inspiring, smooth and sophisticated atmosphere in which to work. I own my sincere thanks and big hug to the "Haematological Family": Karri Penttilä, M.D., PhD., Päivi Lehtonen, M.D., Marja Pyörälä, M.D., PhD., and Urpu Salmenniemi, M.D., PhD., for understanding and support. I am very thankful to Jarkko Magga, M.D., PhD., for being my "personal trainer" with computers.

Above all, I am very grateful to all those patients who participated in this study. I hope that these results will help in further studies that try to find better cure for their malignant disease.

But there is life beyond scientific writing and friends make life worth living! I have a great privilege to be associated in many so called "organisations": Trio Törkeät, Seestyneet, Telomeerit, 10th floor ladies, etc. I want warmly thank all my friends for supporting me, especially in those "dark" moments. It is a great pleasure to know you and spend time with you among many fascinating activities.

My final thanks go to those I love most. My warmest thanks belong to my parents Eila and Reima Kuittinen for their constant love, encouragement and incredible support! You have created me excellent possibilities for life. My father died in August 2004 and did not see this thesis to reach its completion, but his wise advice and tender smile have guided me. I also warmly thank my sister Sari Kuittinen-Tihilä and her family, Kai, Laura and Max, for many delightful moments together. You have kept my feet on the ground during this project. I wish to thank my godparents, Raili and Antero Mattlar, for their kindness and support.

This work was financially supported by grants from the Finnish Society of Haematology, Blood Research Foundation, Cancer Foundation of North Savo, Aleksanteri Mikkonen Foundation, and EVO funding from Kuopio University Hospital.

Kuopio, March 2006

Taru Kuittinen

PRINCIPAL ABBREVIATIONS

AA	Ann Arbor
ANP	Atrial natriuretic peptide
ASCT	Autologous stem cell transplantation
BM	Bone marrow
BNP	Brain natriuretic peptide
CHF	Congestive heart failure
CR	Complete remission
CY	Cyclophosphamide
DLBCL	Diffuse large B-cell lymphoma
ECG	Electrocardiography
Echo	Echocardiography
FL	Follicular lymphoma
G-CSF	Granulocyte colony -stimulating factor
GM-CSF	Granulocyte-macrophage colony -stimulating factor
HDT	High-dose therapy
HPC	Haematopoietic progenitor cell
IPI	International Prognostic Index
LA	Left atrial
LDH	Lactate dehydrogenase
LV	Left ventricular
LVEF	Left ventricular ejection fraction
MCL	Mantle cell lymphoma
MRI	Magnetic resonance imaging
NHL	Non-Hodgkin's lymphoma
OS	Overall survival
PBPC	Peripheral blood progenitor cell
PR	Partial remission
RVG	Radionuclide ventriculography
SCT	Stem cell transplantation
T-NHL	T-cell non-Hodgkin's lymphoma
TRM	Treatment-related mortality
TTPF	Time to peak filling
WHO	World Health Organisation

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which will be referred to their Roman numerals. Some unpublished data are presented.

I **Kuittinen** T, Nousiainen T, Halonen P, Mahlamäki E, Jantunen E. Prediction of mobilisation failure in patients with non-Hodgkin's lymphoma. Bone Marrow Transplant 33: 907-912, 2004.

II Jantunen E, **Kuittinen** T, Nousiainen T. Is chemotherapy scoring useful to predict progenitor cell mobilisation in patients with non-Hodgkin's lymphoma? Bone Marrow Transplant 32: 569-573, 2003.

III Jantunen E, **Kuittinen** T, Nousiainen T. BEAC or BEAM for high-dose therapy in patients with non-Hodgkin's lymphoma: a single centre analysis on toxicity and efficacy. Leuk Lymphoma 44: 1151-1158, 2003.

IV **Kuittinen** T, Husso-Saastamoinen M, Sipola P, Vuolteenaho O, Ala-Kopsala M, Nousiainen T, Jantunen E, Hartikainen J. Very acute cardiac toxicity during BEAC chemotherapy in non-Hodgkin's lymphoma patients undergoing autologous stem cell transplantation. Bone Marrow Transplant 36: 1077-1082, 2005.

V **Kuittinen** T, Jantunen E, Vanninen E, Mussalo H, Vuolteenaho O, Ala-Kopsala M, Nousiainen T, Hartikainen J. Cardiac effects within three months of BEAC high-dose therapy in non-Hodgkin's lymphoma patients undergoing autologous stem cell transplantation. Eur J Haematol, in press.

VI **Kuittinen** T, Wiklund T, Remes K, Elonen E, Lehtinen T, Kuittinen O, Leppä S, Putkonen M, Räty R, Turpeenniemi-Hujanen T, Nousiainen T, Jantunen E. Outcome of progressive disease after autologous stem cell transplantation in patients with non-Hodgkin's lymphoma: a nation wide survey. Eur J Haematol 75: 199-205, 2005.

CONTENTS

1. INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is not a single disease, but rather a group of closely related B- and T-cell malignancies affecting the lymphatic system and ranging from predominantly indolent to highly aggressive lymphomas (Harris et al. 1999). NHLs vary with respect to their molecular features, genetics, clinical presentation, treatment approaches and outcome. Furthermore, therapeutic options are extremely varied with regard to efficacy, toxicity and costs. The incidence of NHL is steadily increasing in the Western World.

NHL is nowadays among the leading indications for high-dose therapy (HDT) supported by autologous stem cell transplantation (ASCT) (Gratwohl et al. 2004). This treatment modality was introduced in an attempt to intensify therapy and to improve cure rates by overcoming dose-limiting myelosuppression. HDT supported by ASCT is currently indicated for chemosensitive, relapsed aggressive NHL and as salvage therapy cures almost 50 % of these patients (Philip et al. 1995). This treatment also improves outcome in patients with mantle cell lymphoma (Dreyling et al. 2005) and relapsed follicular lymphoma (Schouten et al. 2003).

Successful ASCT requires the infusion of a sufficient number of haematopoietic progenitor cells (HPCs) (Stiff et al. 1987, Weaver et al. 1997). Peripheral blood progenitor cells (PBPCs) are accepted as the optimal stem cell source for patients undergoing ASCT (Appelbaum et al. 1996, To et al. 1997, Gratwohl et al. 2004). However, under steady state haematopoiesis the number of HPCs is low in peripheral blood and therefore HPCs are collected by aphaeresis procedure from blood after mobilisation. Classically granulocyte colony-stimulating factor (G-CSF) has been used alone or in combination with myelosuppressive chemotherapy for HPC mobilisation. Unfortunately a significant proportion of NHL patients fail to mobilise a sufficient number of HPCs to proceed to HDT.

Increasing dose intensity certainly has the potential to improve response and cure rates, but this leads inevitably to increased toxicity. The most common toxic effects of HDT

regimens and causes for transplant-related morbidity are myelosuppression, diarrhoea and mucositis. The use of high-dose cyclophosphamide (CY) as part of HDT has been considered to be the main cause of cardiac toxicity. However, the precise mechanisms of high-dose CY related cardiac toxicity are unknown. This toxicity may present clinically as arrhythmias, myopericarditis, and congestive hearth failure during or after HDT (Gottdiener et al. 1981, Cazin et al. 1986, Kupari et al.1990a, Murdyck et al. 2001).

Relapse or progressive disease is the most common reason for treatment failure after ASCT in NHL patients. Although the general impression is that the prognosis of patients who progress after ASCT is poor, the individual prognosis may vary considerably (Vose et al. 1992). Emerging data indicate that an increasing number of these patients can be reinduced into remission. However, limited data are available on factors predicting treatment response and outcome in this patient population.

This study was undertaken to examine different aspects of HDT supported by ASCT in adult NHL patients: prediction of HPC mobilisation, toxicity of HDT with special reference to cardiac toxicity and prognosis of NHL patients experiencing progressive disease after ASCT. First of all, factors affecting HPC mobilisation in NHL patients mobilised with intermediate-dose CY and G-CSF were evaluated. The interest was to create a mathematical model to help in the prediction of mobilisation failure. Furthermore, the toxicity of two commonly used HDT regimens, BEAC and BEAM was compared. Because cardiac toxicity related to high-dose CY is a potentially fatal complication of HDT, magnetic resonance imaging, radionuclide ventriculography and natriuretic peptide measurements were prospectively used to assess cardiac effects of BEAC chemotherapy. Finally, factors predicting treatment response and outcome among NHL patients experiencing progressive disease after ASCT to optimise management in this clinically challenging patient group were evaluated.

2. REVIEW OF THE LITERATURE

2.1. Non-Hodgkin's lymphoma

NHLs are a heterogenous group of malignancies of the lymphoid system. They are neoplasms that originate from precursors at various stage of lymphocyte development, most often from B- or T-lymphocyte lineage and rarely from natural killer (NK)-cell lineage. They exhibit distinct morphological, immunophenotypic, molecular and genetic features allowing specific classification. In addition, clinical presentation, treatment approaches and outcome vary markedly among different subtypes. Clinically NHLs are classified as indolent (low-grade), intermediate and aggressive (high-grade) tumours (Harris et al. 1999). The majority of new cases (50 %) are intermediate-grade lymphomas, followed by low-grade (30-35 %) and high-grade (10 %) lymphomas.

Treatment of NHLs is based on the evaluation of lymphoma subtype and risk factors. In addition, precise staging of NHL is a prerequisite for the selection of a suitable therapeutic regimen and influences the likelihood of its success. Further, the ultimate goal of the therapy may differ according to the histological subtype. Despite many effective treatment modalities disease relapse remains a problem in NHL patients.

The world-wide incidence of NHL rose from 1970s to the 1990s. Even though the incidende did already level off, it has recently started to rise again. The number of new cases registered in Finland is about 1000 per year. In 2003, NHL was in the sixth most frequent cancer among men and seventh in women in our country.

2.1.1. Histological classification

For a decade or so, NHLs were classified according to the International Working Formulation which was primarily based on morphologic appearance and, to a lesser extent, clinical behaviour. The Revised European-American Lymphoma classification (REAL) distinquished lymphomas not only by histology, but by immunophenotype, genetic and clinical characteristics (Harris et al. 1994). This system was later modified and is nowadays universally accepted as the World Health Organizisation (WHO) classification (Harris et

al.1999) (Table 1). The most common forms of NHL are diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle cell lymphoma (MCL) and T-cell NHL (T-NHL).

Table 1. The World Health Organization (WHO) classification of lymphoid neoplasms.

Classification	Percentage of total cases
B –Cell Neoplasms	90 %
a) Precursor B-cell neoplasms	
Precursor B lymphoblastic leukemia/lymphoma	1-2 %
b) Mature (peripheral) B-cell neoplasms	
Diffuse large B-cell lymphoma	31 %
Mediastinal large B-cell lymphoma	
Primary effusion lymphoma	
Follicular lymphoma	22 %
Mantle cell lymphoma	6 %
Nodal marginal zone B-cell lymphoma	1.8 %
Extranodal marginal zone B-cell lymphoma of MALT type	7.6 %
Lymphoplasmacytic lymphoma	1.2 %
Burkitt's lymphoma/leukemia	2.5 %
B-cell chronic lymphocytic leukemia/ small lymphocytic lymphoma	6.7 %
B-cell prolymphocytic leukemia	1 %
Hairy cell leukemia	<1 %
Plasma cell myeloma/plasmacytoma	35 %
Splenic marginal zone B-cell lymphoma	<1 %
c) B-cell proliferation of uncertain malignant potential	
Lymphomatoid granulomatosis	<1 %
Posttransplantation lymphoproliferative disorder, polymorphic	<1 %

T- and NK-cell neoplasms	10 %
a) Precursor T-cell neoplasms	
Precursor T-cell lymphoblastic leukemia/lymphoma	1.7 %
b) Mature (peripheral) T-cell neoplasms	7.6 %
Anaplastic large-cell lymphoma, T/null cell/ primary cutaneus type	2.4 %
Peripheral T-cell lymphoma, unspecified	3.4 %
Enteropathy-type T-cell lymphoma	<1 %
Angioimmunoblastic T-cell lymphoma	1.2 %
Primary cutaneus anaplastic large cell lymphoma	<1 %
Mycosis fungoides /Sezary syndrome	<1 %
Adult T-cell lymphoma/leukemia	<1 %
Hepatosplenic T-cell lymphoma	<1 %
Subcutaneus panniculitis-like T-cell lymphoma	<1 %
Extranodal T/NK-cell lymphoma, nasal type	<1 %
T-cell granular lymphocytic leukemia	<1 %
T-cell prolymphocytic leukemia	<1 %
Aggressive NK-cell leukemia	<1 %
c) T-cell proliferation of uncertain malignant potential	
Lymphomatoid papulosis	<1 %

Hodgkin's lymphoma

Nodular lymphocyte –predominant Hodgkin's lymphoma
Classical Hodgkin's lymphoma
Nodular sclerosis Hodgkin's lymphoma (grades 1 and 2)
Lymphocyte-rich classical Hodgkin's lymphoma
Mixed cellularity Hodgkin's lymphoma
Lymphocyte depletion Hodgkin's lymphoma

2.1.2. Prognostic factors

The outcome of NHL patients is highly variable and the histology is the major determinant of treatment outcome and prognosis. However, even within the same histological subtypes, patients vary considerably with regard to outcome. The International Prognostic Index (IPI) was developed using data from a large number of similarly treated patients with DLBCL (A predictive model for aggressive non-Hodgkin's Lymphoma 1993). Based on age, performance status, serum lactate dehydrogenase (LDH), number of extranodal sites of NHL involvement and Ann Arbor (AA)-stage (Table 2), patients could be separated into prognostically distinct groups. These parameters measure the invasive potential and patient's immune response to the lymphoma as well as their ability to tolerate treatment.

Subsequently this original 5-grade IPI was modified as an age-adjusted IPI (aaIPI) and was recommended for patients under 60 years of age (The non-Hodgkin's Lymphoma Classification Project 1997). Although IPI was initially proposed for aggressive NHL histologies, a number of studies have suggested that it can also be used within indolent histologies (Lopez-Guillermo et al. 1994). In addition, IPI has predictive value also in patients with T-NHL (Lopez-Guillermo et al. 1998). More recently, a similar prognostic model was developed for FL, referred to as the Follicular Lymphoma IPI (FLIPI) (Solal-Celigny et al. 2004). In addition to lymphoma subtype and accurate staging of the disease, the treatment of NHL is guided by the presence or absence of adverse prognostic factors.

Table 2. International Prognostic Index (IPI).

Factor	Adverse prognosis
Age	$\geq$ 60 years
Ann Arbor stage	III-IV
Serum LDH	above normal
Number of extranodal sites of involvement	$\geq$ 2
Performance status according to WHO	$\geq$ 2

Abbreviations: LDH, lactate dehydrogenase; WHO, World Health Organisation

At present, pending the completion of molecular response profile studies, the clinical factors of the IPI are still used to identify patients at increased risk of standard treatment failure for whom experimental therapy would be appropriate (Staudt et al. 2003). However, just recently DNA microarray analyses that identify genes that are either overexpressed or underexpressed by the malignant cells, have further distinquished patients into different risk groups even within same NHL histologies (Alizadeh et al. 2000, Rosenwald et al. 2003, Bea et al. 2005).

2.2. Autologous stem cell transplantation

ASCT includes dose-escalated chemotherapy alone or in combination with total body irradiation in order to overcome tumor resistance and to achieve long-term survival. After HDT, HPCs collected from peripheral blood or bone marrow (BM), are infused to the patient for haematopoietic rescue.

Initially ASCT was performed with the support of BM cells. ASCT was tested not only in haematolocical malignancies but also in numerous solid tumours. In 1986 the first successful ASCT by PBPC support was performed (Kessinger et al. 1986). Furthermore, in the early 1990's, peripheral blood replaced BM as the preferred source of HPC support in autologous setting because of more rapid engraftment of neutrophils and platelets and thus lesser toxicity and reduced need for supportive care (Craig et al. 1993, Bensinger et al. 1995, Schmitz et al. 1996, To et al. 1997).

NHL patients with aggressive histology who fail to respond to their initial treatment will eventually die of their disease unless a complete remission (CR) is achieved with salvage therapy (Verdonck et al. 1992, Mounier et al. 1998). The randomised Parma-study demonstrated a survival advantage for HDT over second-line conventional chemotherapy in patients with relapsed, histologically aggressive NHL (Philip et al. 1995). This study has been accepted as "proof of principle". Since then, the last 10 years has been a remarkable increase in the number of patients receiving HDT supported by ASCT. NHL is currently the second most common indication for HDT supported by ASCT and according to European Bone Marrow Transplantation registry data, 4023 patients received ASCT for NHL in 2003 (Gratwohl et al. 2004). In 1990-2003, ASCT was performed for 542 adult NHL patients in Finland (Jantunen et al. 2006).

2.2.1. Indications

In general, the prerequisite for ASCT has been a chemosensitive disease and no severe co-morbidities. With recognition of the fact that the single most important prognostic factor for outcome was remission status at the time of HDT, ASCT is no longer considered appropriate for patients with progressive disease (Kewalramani et al. 2000, Vose et al.

2001). In fact, patients with progressive, aggressive NHL subtype have response rates of less than 15 % to any salvage therapy, and fewer than 5 % of them are likely to have a favourable response to ASCT.

2.2.1.1. Diffuse large B-cell NHL

Although about 50 % of patients with DLBCL will be cured of their disease by initial combination chemotherapy, standard-dose chemotherapy may salvage no more than 10 % of patients with relapsed or refractory disease (Shipp et al. 1995, Mounier et al. 1998). Several clinical trials have been published in an attempt to clarify the optimal timing and define a risk-adapted strategy for maximal benefit of ASCT in DLBCL. The Parma-study, which randomised NHL patients with relapsed, aggressive histology to either ASCT or to conventional chemotherapy with DHAP (dexamethasone, cisplatin, cytarabine) plus involved- field radiation, showed a benefit for patients treated in ASCT arm with better 5-year event-free survival (EFS; 46 % vs. 12 %) and overall survival (OS; 53 % vs. 32 %) over conventional chemotherapy (Philip et al. 1995). According to this randomised trial, ASCT is indicated for salvage therapy for relapsed chemosensitive DLBCL.

Because of the poor outcome with standard chemotherapy in patients with intermediate-high or high IPI with expected 5-year disease-free survival (DFS) of less than 30 %, there has been great interest to use ASCT as a consolidation therapy in the first remission. One randomised trial showed evidence that early ASCT is superior to standard chemotherapy in terms of EFS, without significant OS advantage (Gianni et al. 1997). Thereafter several randomised studies have failed to shown an advantage of early ASCT (Verdonck et al. 1995, Kluin-Nelemans et al. 2001, Gisselbrecht et al. 2002, Kaiser et al. 2002). Two randomised trials have, however, shown the OS advantage in ASCT arm over conventional chemotherapy (Haioun et al. 2000, Milpied et al. 2004). Thus, the role of ASCT as consolidation therapy in first remission for patients with DLBCL remains undefined. Moreover, in these studies the conventional chemotherapy-arm has not included monoclonal antibody (rituximab), which has been shown to increase OS rates in DLBCL (Coiffier et al. 2002).

2.2.1.2. Follicular lymphoma

ASCT has been shown to be superior to standard chemotherapy in patients with relapsed FL in terms of PFS and OS in a randomised trial (Schouten et al. 2003). In addition, several retrospective evaluations have been conducted on this item concluding that prolonged relapse free time can be achieced with ASCT (Freedman et al. 1996, Apostolidis et al. 2000).

Because advanced stage FL (AA $\geq$ III) almost invariably relapses after initial therapy, several studies have evaluated the use of ASCT as a consolidation therapy in the first remission after conventional chemotherapy. A multicenter, prospective trial demonstrated that ASCT could be routinely offered to young (< 60 y) poor-risk FL patients. The projected DFS at 4-years was 85 % in this patient population (Ladetto et al. 2002). In fact, there are only two published, randomised trials focusing to the use of ASCT as part of first-line consolidation. The GOELAMS study showed that patients receiving ASCT compared to those receiving chemotherapy plus interferon had higher response rates (69 % vs. 81 %) and longer median EFS. This did not, however, translate into a better OS due to an excess of secondary malignancies after ASCT. Thus, ASCT can not be considered as the standard first-line treatment for FL patients younger than 60 y with high tumor burden (Deconinck et al. 2005). In another randomised study myeloablative radiochemotherapy followed by ASCT was compared to interferon maintenance. In patients who underwent ASCT, a 5-year PFS rate was significantly higher (64.7 % vs. 33.3 %). Longer follow-up is necessary to determine the effect of ASCT on OS (Lenz et al. 2004).

In about 30 %-50 % of patients with FL the disease eventually transforms to more aggressive histological subtypes, intermediate or high-grade NHL. Two retrospective studies indicate that ASCT is a viable treatment option in these patients (Foran et al. 1998, Williams et al. 2001). In the latest retrospectice study on relapsed FL patients including also patients with transformed disease, ASCT was superior according to OS compared to conventional chemotherapy (Andreadis et al. 2005). No randomised trials are available in this patient group.

2.2.1.3. Mantle cell lymphoma

No curative therapy currently exists for advanced stage MCL and patients invariably relapse after the initial therapy. In the first remission promising results have been shown with ASCT (Andersen et al. 2003, Vandenberghe et al. 2003, Geisler et al. 2004). In the prospective Milan study, monoclonal antibody was combined to HDT and this treatment arm showed OS advantage over historical controls (Gianni et al. 2003). Just recently the first randomised study has been published on this item. Early consolidation with ASCT in first remission in advanced stage MCL significantly prolonged PFS compared to interferon maintenance. Longer follow-up is needed to determine the effects on OS (Dreyling et al. 2005).

2.2.1.4. T-cell lymphoma

Despite aggressive therapy, the long-term prognosis of most T-NHL patients is unfavourable (Gisselbrecht et al. 1998, Lopez-Guillermo et al. 1998). Several retrospective analyses have documented the feasibility of ASCT with long-term survival rates similar to transplanted B-NHL patients (Blystad et al. 2001, Rodriquez et al. 2001, Song et al. 2003, Jantunen et al. 2004). The first prospective study of ASCT in patients with peripheral T-NHL compared to historical controls demonstrated that ASCT is feasible and effective in first-line consolidation even though survival benefit was not established (Reimer et al. 2004). At present no randomised trials of ASCT in T-NHL are available.

2.2.2. Mobilisation of haematopoietic progenitor cells

Stem cells are regarded as clonogeneic cells capable of self-renewal and multilineage differentation. Progenitor cells are oligo-lineage cells that are already more restricted in their differentation potential and not capable for self-renewal (Weissman et al. 2000). In fact, the words stem cell and progenitor cell are often applied as synonyms in literature. The expression of CD34-antigen is commonly used in clinical practise as a surrogate marker for haematopoietic stem and progenitor cells (Seggewiss et al. 2003). The percentage of $CD34^+$ cells in peripheral blood is only about 0.06 % (Fruehauf et al. 1995).

However, this number can be considerably increased by a variety of stimuli. The use of PBPCs has led to reduced morbidity and mortality as well as shorter hospitalisation with reduced resource utilisation compared to use of BM stem cells in transplantation procedure (Schmitz et al. 1996, Glaspy et al. 1999, Vellenga et al. 2001).

2.2.2.1. Biology of HPC mobilisation

Some studies have challenged the dogma that all HPCs express CD34 antigen (Zanjani et al. 1998, Petit et al. 2002). The latest finding has demonstrated that only one class of murine stem cells exists, although in two functional states distinguishable by CD34 expression (Sato et al.1999, Lapidot and Petit 2002). According to this model, $CD34^-$ stem cells are quiescent and can be activated by different stimuli to generate a $CD34^+$ cell population with a high engraftment potential.

Expression of the G-CSF receptor on stem cells is not required for their mobilisation. G-CSF or myelosuppressive therapy acts via secondary pathways, including the chemokine stromal-derived factor-1 (SDF-1) and its receptor CXCR4 (Pelus et al. 2002). BM stromal cells produce SDF-1, whose receptor is presented on $CD34^+$cells (Aiuti et al. 1997). One of the predominant stimuli for the trafficking of HPCs to and their retention within the marrow occurs through the trophic influence of SDF-1 (Flomenberg et al. 2005). The SDF-1 agonist CTCE0021 competes with stroma-bound SDF-1 for CXCR4 binding. In addition, it has been demonstrated that the activation of the matrix metalloprotease-9 (MMP-9) is a decisive checkpoint for the mobilisation of HPCs as it promotes the recruitment of these cells into peripheral blood (Pelus et al. 2004).

2.2.2.2. Definitions of HPC mobilisation

Because the number of infused $CD34^+$ cells correlates with the rate of haematopoietic reconstitution, the adequate amount of HPCs is a prerequisite for the feasibility of HDT. The number of HPCs is quantified by the number of $CD34^+$ cells infused per kilogram of body weight. The engraftment is measured by recovery of absolute neutrophil count > 0.5 x 10^9/l and platelet count > 20 x 10^9/l after ASCT (Weaver et al. 1997, Ketterer et al. 1998).

Succesfull mobilisation. Studies have shown that > 2.0 x 10^6 $CD34^+$ cells /kg results in reliable engraftment within 14 days after HPC infusion (Haas et al. 1994, Ketterer et al. 1998). In the majority of the patients the required cell dose can be collected with one or two collections. Transplanting patients with HPC dose of $\geq$ 5.0 x 10^6/kg $CD34^+$ cells lead to neutrophil engraftment at 8-10 days after transplant and platelet recovery at 10-12 days (Weaver et al. 1997).

Mobilisation failure. About 5-30 % of the patients with NHL are difficult to mobilise or fail HPC mobilisation with current methods (Watts et al. 1997, Stiff et al. 1999, Sugrue et al. 2000). Those patients who do not achieve the optimal dose HPC yield ($\geq$ 2.0 x 10^6/kg $CD34^+$ cells) in one to two aphaeresis to proceed to HDT are called poor mobilisers (Stiff et al. 1999). Up to 30 % of patients do not engraft platelets if they receive only 1.0 x 10^6/kg $CD34^+$ cells (Gandhi et al. 1999, Stockerl-Goldstein et al. 2000) while almost any dose of $CD34^+$ cells > 1.0 x 10^6/kg leads to rapid and consistent neutrophil engraftment (Ketterer et al. 1998, Stiff et al. 1999). A general consensus exists that patients receiving < 2.0 x 10^6/kg $CD34^+$ cells are at risk for delayed haematopoietic recovery after HDT (Weaver et al. 1995, Moskowitz et al. 1998, Glaspy et al. 1999).

2.2.2.3. Factors affecting HPC mobilisation

Several factors have been shown to have negative impact to HPC mobilisation. Previous stem cell toxic chemotherapy, high number of previous chemotherapy regimens and/or cycles and long time from diagnosis to PBPC collection appear to be the most significant predictors for poor mobilisation (Ketterer et al. 1998, Stiff et al. 1999). Combination of fludarabine and CY has been found especially toxic to stem cells (Laszlo et al. 2000, Tournilhac et al. 2004). Factors predicting poor HPC mobilisation in NHL patients are presented in Table 3. All factors associated with poor mobilisation are not known since some heavily pretreated patients or patients with marrow infiltration or fibrosis mobilise well, whereas some healthy stem cell donors mobilise poorly (Roberts et al.1995).

Table 3. Risk factors for poor HPC mobilisation in NHL patients.

1. Prior radiotherapy to BM-bearing sites (Haas et al. 1994, Bensinger et al. 1995)
2. Prior stem cell cytotoxic therapy: fludarabine, high-dose Ara-C, platinum compounds, carmustine, nitrogen mustard (Drake et al. 1997, Ketterer et al. 1998, Laszlo et al. 2000, Vantelon et al. 2000)
3. Number of prior chemotherapy cycles (> 6) (Bensinger et al. 1995, Seggewiss et al. 2003)
4. Number of prior chemotherapy regimens (> 2) (Ketterer et al.1998, Stiff et al. 1999, Sugrue et al. 2000).
5. BM involvement (Bensinger et al. 1995, Perry et al. 1998)
6. Time from diagnosis to HPC collection > 12 months (Ketterer et al. 1998).
7. Exposure to any chemotherapy > 6 months (Ketterer et al. 1998)
8. Indolent NHL subtype (Perry et al. 1998)

Abbreviations: NHL, non-Hodgkin's lymphoma; BM, bone marrow; WBC, white blood cell; PBPC, peripheral blood progenitor cell

Drake et al. (1997) proposed a chemotherapy scoring system for previous chemotherapy predicting HPC mobilisation in patients with NHL, multiple myeloma or Hodgkin's disease. This scoring is presented in Table 4. Exposure to toxicity factor (TF) 4 drugs, especially melphalan, has turned out to be a significant independent factor predicting poor HPC mobilisation in patients with haematological malignancies (Kotasek et al. 1992, Goldschmidt et al. 1997).

Table 4. The chemotherapy scoring by Drake et al. (1997).

Toxicity factor	Drug
0	prednisolone, dexamethasone
1	vincristine, interferon-α, bleomycin, vinblastine
2	cyclophosphamide, anthracyclines, cisplatin, etoposide
3	chlorambucil, procarbazine
4	melphalan, carmustine, lomustine, mechlorethamine

2.2.2.4. Mobilisation methods

Classical strategies for HPC mobilisation include administration of G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) alone or in combination with myelosuppressive chemotherapy (Demirer et al. 1996, Gazitt 2000).

Cytokine(s). In 1988, two haematopoietic cytokines, GM-CSF (molgramostim) and G-CSF (filgrastim, lenograstim) showed their efficacy to mobilise HPCs to the blood stream (Haas et al. 1990, Armitage et al. 1998). No difference in their mobilisation ability has been found (Grigg et al. 1995, Demirer et al. 2002, Gazitt et al. 2002). The use of cytokines alone for HPC mobilisation in autologous setting might be beneficial by avoiding the risks of myelosuppressive chemotherapy.

Chemotherapy plus cytokine(s). This is currently the most commonly used mobilisation method. Several studies have shown that myelosuppressive chemotherapy followed by a cytokine yields higher numbers of autologous $CD34^+$ cells than cytokines alone (Demirer et al. 1996, Russell et al. 1998, Narayanasami et al. 2001). Single-agent CY in variable doses (1.5-7 g/m^2) is a widely applied mobilisation regimen (Rowlings et al. 1992, Watts et al. 1997, Fitoussi et al. 2001, Pavone et al. 2002). Also chemotherapy given as part of disease-spesific NHL therapy can be used for HPC mobilisation (McQuaker et al. 1999, Moskowitz et al. 1999, Tarella et al. 1999, Aurlien et al. 2001). CY plus G-CSF has mobilised $CD34^+$ cells more effectively than GM-CSF plus G-CSF (Koc et al. 2000). After chemotherapy, the mobilisation yield is not dependent on the G-CSF or GM-CSF dose (Martin-Murea et al. 1998).

2.2.2.5. Management of poor mobilisers

Remobilisation of HPCs. Remobilisation has included the use of chemotherapy plus cytokine, or cytokines alone at standard or escalated doses (Stiff et al. 1999, Seggewiss et al. 2003). If the cytokine is used alone in this setting, a dose escalation is recommended (G-CSF > 10 μg/kg/day s.c.) (Kobbe et al. 1999, Stiff et al. 1999). Remobilisation with cytokine alone might be the preferred method based on similar $CD34^+$ cell yields and

shorter hospitalisation compared to mobilisation with cytokine plus chemotherapy (Weaver et al. 1998, Glaspy et al. 1999, Watts et al. 2000).

BM harvesting. Poor mobilisers undergoing subsequently ASCT supported by BM cells have shown delayed engraftment leading to increased treatment-related mortality (TRM) and morbidity (Watts et al. 1998). In general, the data suggest a limited role for BM harvesting to supplement an inadequate HPC mobilisation (Stiff et al. 1999). On the other hand, one prospective trial has confirmed that G-CSF-mobilised BM progenitor cells induces effective multilineage haematopoietic recovery after HDT and could be safely used in patients with poor HPC mobilisation (Lemoli et al. 2003).

HPC mobilisation might be improved by molecules capable of interfering with the mechanisms regulating haematopoietic stem cell tracking. These strategies have been used for poor mobilisers but subsequently also in first-line mobilisation. Early- and late-acting cytokines have been explored as HPC mobilisers, including erythropoietin (Kessinger et al. 1995), interleukin-6 (Pettengell et al. 1995) and FLT-3 ligand (Lebsack et al. 1997, Stiff et al. 1999). An improved $CD34^+$ cell mobilisation might also be achieved by combinations of cytokines such as interleukin-3 plus G-CSF (Orazi et al. 1992). Stem cell factor, a cytokine that acts on primitive haematopoietic stem cells, as added to G-CSF may also provide a more effective mobilisation than G-CSF alone (To et al. 2003, Dawson et al. 2005). Human growth-hormone (GH) is a pleiotropic cytokine targeting a variety of nonhaematopoietic and haematopoietic cells by binding to a specific receptor. Addition of GH to G-CSF allows a sufficient HPC mobilisation (Carlo-Stella et al. 2004). More recently, a pegylated G-CSF (pegfilgrastim) has become available. Emerging evidence suggest that pegfilgrastim may be employed as a single dose s.c. injection in HPC mobilisation combined with chemotherapy (Ng et al. 2005).

Chemokine-receptor agonists lead to a rapid and substantial HPC mobilisation. Both SDF-1 and CXCR4 undergo degradation through the action of neutrophil derived proteases released as a consequence of the neutrophil proliferation seen after G-CSF administration, contributing to the HPC mobilisation produced by the drug (Lapidot et al. 2002). AMD-3100 is a partial CXCR4 antagonist and reversibly inhibits SDF-1/CXCR4 binding (Devine

et al. 2004). A prospective study revealed that AMD3100 was well tolereted and resulted in rapid HPC mobilisation in NHL patients when administrated daily s.c. (Devine et al. 2004). AMD3100 plus G-CSF has been effective and superior to G-CSF alone for HPC mobilisation in adult NHL patients (Flomenberg et al. 2005). Several new compounds are under development and will be shortly on phase I-II trials (Pelus et al. 2004).

2.2.3. Aphaeresis of haematopoietic progenitor cells

The goal of HPC collection is to receive an adequate number of $CD34^+$ cells with as few aphaeresis procedures as possible. Therefore timing to start HPC aphaeresis is important and several criteria have been recommended. A white blood cell (WBC) count or mononuclear cell count obtained immediately prior to HPC collection showed only a weak correlation with the number of $CD34^+$ cells aphaeresed (To et al. 1998). On the other hand, a good correlation was found between the absolute numbers of blood $CD34^+$ cells and HPC aphaeresis yield (Armitage et al. 1997, Remes et al. 1997). With peripheral blood $CD34^+$ cell counts > 20 x 10^6/l, a single collection yield was > 2.0 x 10^6 $CD34^+$cells/kg in 94 % of patients (Krieger et al. 1999). In the majority of the patients the required minimal safe cell dose ($\geq$ 2.0 x 10^6/kg $CD34^+$ cells) to procede to ASCT can be collected in one or two collections.

Vascular access for aphaeresis procedure requires a continuous blood flow of 60-100 ml/min in adults. Whenever possible, it is better to use peripheral venous route to avoid the complications associated with a central venous line cannulation. A stiff, dual lumen catheter placed in a subclavian or internal jugular vein also provides adequate flow, but catheter related complications like pneumo-and haemothorax, infections and thrombosis are possible.

The number of HPCs collected with an aphaeresis depends on the concentration of HPCs in the peripheral blood, the blood volume processed and the collection efficiency of the instrument. Conventionally two to three blood volumes are processed or the procedure is limited to a fixed period of time such as 4-6 hours.

2.2.4. High-dose therapy

The most important issue in a case of HDT in autologous setting is to maximise antitumour activity with acceptable toxicity (Mounier and Gisselbrecht 1998). The optimal HDT regimen in NHL is not known since no randomised studies have addressed this issue. The most commonly used HDT regimens are BEAM and CBV. BEAC, in which melphalan is substituted by CY compared to BEAM, is also widely applied. In Table 5 the most commonly applied HDT regimens in NHL patients are presented. In fact, there are several BEAM variations, which differ from each other according to the dose of etoposide (VP-16), carmustine (BCNU) or cytarabine (Ara-C). The dose of melphalan is most often 140 mg/m^2 in NHL patients. Many centres favour melphalan-based HDT when prior HPC mobilisation has been performed by CY-containing regimen. Furthermore, for BEAC the CY dose is usually 6000 mg/m^2. The administration of total-body irradiation (TBI) as a part of HDT in ASCT is controversial, because some centres have reported improved outcome (Horning et al. 1994) and others documented increased acute and long-term toxicity (Weaver et al. 1994).

Table 5. Commonly used HDT regimens in NHL patients.

Regimen	Drugs and total doses
BEAM	Carmustine 300-600 mg/m^2, etoposide 400-1200 mg/m^2, cytarabine 800-1600 mg/m^2, melphalan 100-140 mg/m^2
CY/TBI	Cyclophosphamide 120 mg/kg, TBI 12 Gy
CBV	Cyclophosphamide 6000-7000 mg/m^2, carmustine 300-600 mg/m^2, etoposide 600-2400 mg/m^2
BEAC	Carmustine 300 mg/m^2, etoposide 300-800 mg/m^2, cytarabine 800 mg/m^2, cyclophosphamide 6000 mg/m^2
BuCY	Busulfan 16 mg/kg, *c*yclophosphamide 120 mg/kg
BuCYVP-16	Busulfan 14 mg/kg, cyclophosphamide 120 mg/kg, etoposide 60 mg/kg
CY/VP-16/TBI	Cyclophosphamide 100 mg/kg, etoposide 60 mg/kg, TBI 12 Gy

Abbreviations: TBI, total-body irradiation; Gy, Gray

Advances in supportive care and the use of PBPC support instead of BM cells have significantly reduced early treatment-related mortality (TRM) over recent years (Ketterer et al. 1999, Schmitz et al. 2004, Lyman et al. 2005). Even though the TRM of ASCT is nowadays less than 5 % in NHL patients, the treatment is still associated with life-threatening complications (van Besien et al. 1995, Jantunen et al. 2006).

2.2.4.1. Toxicity of high-dose therapy

The toxicity profile of ASCT is related to the HDT regimen applied (Mounier and Gisselbrecht 1998, Salar et al. 2001). While haematopoioetic toxicity of HDT is no longer dose-limiting, damage to other tissues has become a clinically important consequence.

Myelosuppression. Infectious complications are the most common cause of morbidity and mortality in the peri-transplant period (Ketterer et al. 1999). The incidence and severity of infections are related to the depth and duration of neutropenia. Gram-positive bacteremia now accounts for 60-70 % of microbiologically documented infections, although the rate of gram-negative infections has increased in some centres (Cullen et al. 2005). Randomised trials have demonstrated that G-CSF accelerates the recovery of neutrophils and reduces the duration of severe neutropenia after HDT (Schmitz et al. 2004, Valteau-Couanet et al. 2005). The administration of G-CSF after HDT significantly reduced the incidence of microbiologically defined infections and duration of neutropenia and led to decreased use of antibiotics and earlier discharge from hospital compared to patients treated without G-CSF.

Mucositis. Mucositis is a common complication after HDT and is also related to the depth and duration of neutropenia. Oral and gastrointestinal mucositis have been associated with increased risk of infections, chemotherapy dose reductions and infection-related deaths (Rapoport et al. 1999, Wardley et al. 2000). Keratinocyte growth factor (palifermin) induces basal cell proliferation and differentation in the oral epithelium and might also reduce the rate of cell loss (Farrell et al. 2002). It has been shown to reduce the duration and severity of oral mucositis after ASCT (Spielberger et al. 2004). The dose-limiting toxicity of melphalan in patients undergoing ASCT is severe mucositis (Samuels and Bitran

1995, Moreau et al. 1999). There is evidence that the use of amifostine (AMI), an organic thioposphate, may ameliorate certain regimen-related toxicities of high-dose melphalan in ASCT setting. In a phase I study this drug was used to permit dose-escalation of high-dose melphalan in BEAM regimen (Phillips et al. 2004). A retrospective study showed that AMI reduced severe mucositis and need for analgesic therapy in ASCT recipients (Capelli et al. 2000).

Diarrhoea. Diarrhoea after HDT may be infectious, a consequence of broad-spectrum antibiotics or most commonly, a direct toxic effect of cancer treatment. Severe diarrhoea causes dehydration and risk for renal failure and thromboembolic events. Furthermore, diarrhoea combined with severe neutropenia commonly leads to gram-negative sepsis with a high incidence of mortality (Ozer et al. 2003). Guidelines for management of treatment-induced diarrhoea have been published (Benson et al. 2004). The main aims of treatment should be to treat dehydration aggressively and to use antibiotics if symptoms persist or if there is accompaning neutropenia.

Organ toxicity. Hepatic veno-occlusive disease (VOD) is a life-threatening but relatively rare complication following ASCT. VOD is a clinical syndrome characterized by painful hepatomegaly, jaundice, ascites, fluid retention, and weight gain. The incidence varies depending on HDT regimen used. The mortality is high in severe cases but recovery from VOD has also been seen in over 70 % of patients (Carreras et al. 1998, Lee et al. 1999). The treatment options are limited but heparin, ursodeoxycholic acid and human tissue plasminogen activator have all been used (Simon et al. 2001, Ruutu et al. 2002). Defibrotide has proven effective in patients with severe VOD (Richardson et al. 2002).

Lung toxicity is a relatively common problem following ASCT (Brockstein et al. 2000). The causes of lung injury or interstitial pneumonitis may be infectious, chemical or idiopathic. Interstitial pneumonitis occurs in about 5-10 % of the patients (Wingard et al. 1988), the etiology of which can often be difficult to discern. TBI-containing regimens might increase the risk (Pecego et al. 1986).

Cardiac toxicity is potentially the most fatal non-haematological side-effect of HDT. The use of high-dose CY as part of HDT has been considered to be the main cause for this

toxicity (van Besien et al. 1995, Schrama et al. 2003). Cardiac toxicity related to high-dose CY is discussed more detailed later (page 36).

Storage of HPCs after aphaeresis is required for ASCT. Dimethylsulphoxide (DMSO) is a favoured agent for HPC cryopreservation protecting the cell from excessive dehydratation. The acute toxic dose of DMSO for humans has not been determined and the dose used varies a lot between different centres (Windrum et al. 2005). DMSO has a variety of cardiovascular effects. A number of authors have noted cardiac arrest or high-degree heart block occurring during or immediately after the infusion of cryopreserved HPCs (Styler et al. 1992, Keung et al. 1994). In both reports, the median time of onset was about 3 h after the completion of stem cell infusion and the cardiac rhythm abnormalities resolved spontaneously within 24h of infusion. On the other hand, there are several studies in which no cardiac rhythm changes in association to DMSO have been documented.

Secondary malignancies. Malignant diseases occurring after ASCT are of particular concern as an increasing number of NHL patients survive and remain free of their lymphoma. Forrest et al. (2003) showed a cumulative risk developing a second malignancy of 11 % at 15 years. It has become clear that 4-15 % of NHL patients will develope secondary myelodysplasia or secondary acute leukemia after ASCT (Howe et al. 2003, Ortega et al. 2005).

2.2.5. Progressive disease after ASCT

Although ASCT is a curative treatment in a significant percentage of NHL patients, disease relapse remains the major cause of treatment failure (Johnsen et al. 1996, Vaishampayan et al. 2002, Buchler et al. 2003). Surprisingly, only few formal studies are available on this issue. In the available studies time from ASCT to relapse, and IPI have been shown to be of prognostic significance (Kewalramani et al. 2003, Paltiel et al. 2003).

A large number of patients can be re-induced into durable CR and occasional patients may be cured. However, treatment options in this setting are often limited because of cumulative toxicities from prior chemotherapeutic agents and lack of haematopoietic

reserves (Buchler et al. 2003). In general, reinduction of remission is the most imminent treatment goal. In many cases consolidation of remission is needed.

2.2.5.1. Reinducing remission

Salvage chemotherapy. The short-time objective of treatment for this entire patient group is to choose an effective and appropriate salvage regimen. The prior chemotherapy has to be taken into account and also NHL histology plays an important role. Cumulative cardiotoxicity limits the use of anthracycline-based regimens and nephrotoxicity is the concern in platinum-containing compounds.

Radiation therapy. NHLs are often radiosensitive and this treatment modality is effective especially in indolent NHL. However, relapsed NHL is seldom localised, and with rare exeptions, radiation can not be considered curative in this context.

Monoclonal antibody therapy with rituximab. Rituximab, an anti-CD20-monoclonal antibody, was introduced into clinical practise at the end of 1990's (Maloney et al. 1994, McLaughlin et al. 1998). As a result of its efficacy and favourable toxicity profile, it has rapidly become a component of standard induction therapy in $CD20^+$ B-cell NHLs (Coiffier et al. 2002). However, experience using rituximab for salvage therapy in patients with progressive disease after ASCT is limited. Prior exposure to rituximab is not a contraindication for retreatment (Davis et al. 2000). Therefore, rituximab in combination with other regimen should be considered as salvage therapy for patients relapsing after ASCT because of acceptable toxicity profile (Pan et al. 2002).

Radioimmunotherapy. Since NHLs are inherently radiosensitive neoplasms, antibody-targeted radiotherapy offers a new opportunity to NHL treatment (Witzig et al. 2003). Radioimmunotherapy (RIT) uses a monoclonal antibody in addition to a radionuclide to deliver radiation to the sites of disease. ^{90}Yttrium-ibritumomab tiuxetan and ^{131}I-tositumomab are radio-labeled monoclonal anti-CD20 antibodies that have shown significant activity in NHL. An advantage of their use includes the higher CR rates than can be achieved with single-agent rituximab (Cheson et al. 2005). Disadvantages include the associated myelosuppression and the inability to administer these drugs in patients who

have extensive BM involvement. Limited data are available on the toxicity and efficacy of RIT after ASCT.

2.2.5.2. Consolidation of remission

Patients achieving remission after salvage therapy might be considered for a second ASCT or allogeneic stem cell transplantation.

Second ASCT. The reharvest of HPCs is not always possible because marrow reserves may have been exhausted or because there might be overt BM involvement by lymphoma. There are only few studies dealing with this matter and data suggest that for selected patients a second ASCT offered an acceptable morbidity and mortality and might even be a curative procedure (De Lima et al. 1997, Lenain et al. 2004).

Allogeneic stem cell transplantation. Allogeneic stem cell transplantation (SCT) has the advantage of providing a tumour-free graft and potentially causing graft-versus-lymphoma effect (GVL). The less myeloablative the conditioning regimen, the more disease eradication is dependent on immunological mechanisms. The current data support the view that GVL effect is strongest for indolent lymphomas and MCL, and limited for DLBCL (Khouri et al. 1999, Maloney et al. 2003, van Besien et al. 2003). Nonmyeloablative or reduced-intensity conditioning (RIC) allogeneic SCT has been studied as an optional therapy for patients failing ASCT (Feinstein et al. 2003, Escalon et al. 2004). Furthermore, current data suggest that durable remission can be obtained after RIC for patients failing ASCT (Branson et al. 2002, Fung et al. 2003) and TRM appears to be lower than after myeloablative allogeneic SCT (Freytes et al. 2004). NHL patients, who have HLA-identical sibling donor, reach CR with salvage treatment and have good performance status, are the best candidates for this treatment option (Goldstone et al. 2002, Freytes et al. 2004).

2.3. Cardiac toxicity of cyclophosphamide

CY is a broadly active antineoplastic and immunosuppressive agent. Like other cytotoxic drugs in cancer therapy CY is frequently used in combination for NHL, multiple myeloma, acute leukemias, neuroblastoma, Ewing's sarcoma, lung, breast, ovarian and endometrial

cancer. Further, high-dose CY (i.e. > 5 g/m^2 administered in fractions or as a single dose) is an essential part of many HDT regimens (Cazin et al. 1979, Gottdiener et al. 1981, Hertenstein et al. 1994) (Table 5, page 31) and is also widely applied for HPC mobilisation in combination with growth factors (Rowlings et al. 1992, Fitoussi et al. 2001, Pavone et al. 2002).

2.3.1. Biological action of cyclophosphamide

CY is a prodrug for one or more bifunctional alkylating agents. It requires metabolic activation by cytochrome P450-related enzymes in the liver (Ayash et al. 1992). A number of its metabolities have alkylating activity, but it is likely that the principle alkylating agent is phosphoramide mustard (Colvin et al. 1981). This metabolite is responsible for the therapeutic activity and most of the overall toxicity of CY. The effects of CY are related to the total amount of metabolities produced rather than to their rate of production (McDonald et al. 2003). Thus, cytotoxicity of CY may be directly modulated by inducing or inhibiting microsomal enzyme activation (DeLeve et al. 1996).

CY acts on cells at any stage, but the effects are usually seen when the cell enters the S-phase and progress through the cell cycle is blocked at the G_2 (premitotic) stage (Colvin et al. 1981). Less than 20 % of CY dose appears unchanged in the urine, the remainder being metabolized by the liver (Fraiser et al. 1991). Potentially life-threating side-effects of CY are myelosuppression, cardiac and liver toxicity, carcinogenicity and pulmonary fibrosis. In addition, severe or irreversible adverse reactions include cystitis and infertility.

2.3.2. Pathogenesis of cardiac toxicity

Although a wide variety of HDT regimens have been used in NHL patients, cardiac toxicity has been associated mostly with high-dose CY containing regimens. The cellular mechanisms of CY related cardiotoxicity are thought to be mediated by the increase in free oxygen radicals and through intracellular phosphoramide mustard affecting endothelium and ion transport mechanisms (Levine et al. 1993). Myocyte damage, interstitial haemorrhage, fibrosis and myocardial oedema are the most common microscopic findings

resulting from this toxic endothelial damage (Appelbaum et al. 1976, Gottdiener et al. 1981, Cazin et al. 1986, Kupari et al. 1990a, Murdych et al. 2001). In addition, it has been proposed that endothelial damage with interstitial transudation leads to decreased cardiac electrical activity in the majority of patients receiving high-dose CY (Gottdiener et al. 1981). Glutathione, repairing oxidized injury of cells, has been shown to protect cardiac myocytes in the animal model of 4-hydroperoxy CY-induced damage (Levine et al. 1993). Autopsy studies have also revealed increased heart weight and noticeable thickening of the left ventricular wall (Appelbaum et al. 1976, Kupari et al. 1990a, Murdych et al. 2001).

2.3.3. Clinical manifestations of cardiac toxicity

Severe cardiac toxicity is an uncommon but potentially fatal side-effect of high-dose CY. Cardiac complications have been documented in several series with an incidence ranging from 0 % to 43 % (Gottdiener et al. 1981, Cazin et al. 1986, Kupari et al. 1990a, Hertenstein et al. 1994, Murdych et al. 2001) and mortality from 2 % to 9 % (Cazin et al.1986, Murdych et al. 2001). Many reasons account for this incidence variability, particularly the co-administration of other chemotherapeutic agents, HDT regimens used and patient selection. In addition, there is evidence that single-agent high-dose CY associated cardiac toxicity is dose- and schedule-dependent and is not related to the cumulative drug dose (Goldberg et al. 1986).

High-dose CY associated cardiotoxicity occurs during or soon after the drug administration, most often within 3 weeks (Gottdiener et al. 1981). In general this toxicity may present as arrhythmias, pericardial effusion, myopericarditis or congestive heart failure (CHF). CHF may manifest clinically as acute or subacute onset with pulmonary congestion, weight gain, oliguria and cardiac dilatation. One study showed that those patients, who died due to acute CHF, maintained nearly normal systolic function until the death in the presence of profound unresponsive hypotension (Lee et al. 1996). Pericardial effusion may be the only manifestation of cardiac toxicity (Kupari et al. 1990b, Braverman et al. 1991) or a part of the clinical picture with cardiac tamponade (Gottdiener et al. 1981, Cazin et al. 1986). High-dose CY induced cardiotoxicity may last from one to six days and despite the

relatively high mortality rate there are no long-term sequelaes or late cardiotoxicity in patients who survive the initial acute event (Gottdiener et al. 1981). Although high-dose CY related cardiac toxicity is usually reversible, in patients who develop progressive severe CHF this complication may lead to death within a few weeks (Gottdiener et al. 1981, Goldberg et al. 1986, Braverman et al. 1991).

Some cardiac events occurring in cancer patients may be related to other clinical conditions (i.e. infections, electrolyte abnormalities, disturbed blood gases, vagal reflex and sympatho-adrenergic drive) and therefore should not always be considered as high-dose CY associated adverse effects.

2.3.4. Methods for monitoring cardiac toxicity

There are two major consensus guidelines for the monitoring of chemotherapy-induced cardiotoxicity (Steinherz et al. 1992, Ritchie et al. 1995). The guidelines have proposed to monitor left ventricular ejection fraction (LVEF) by radionuclide ventriculography (RVG) or two-dimensional echocardiography (2D echo) during and shortly after chemotherapy. These guidelines have focuced on anthracycline related cardiotoxicity while no spesific data on high-dose CY have been presented. In addition, no randomised studies are available comparing these two methods in evaluating cardiac dysfunction. In clinical practise patient history, physical examination, X-ray and electrocardiography (ECG) are also very important tools.

Subclinical cardiotoxicity is described as any alteration of functional or biochemical values from baseline measured by different diagnostic techniques. Several studies have reported subclinical cardiotoxicity in the form of asymptomatic left ventricular (LV) dysfunction, accompanied by changes of electrocardiographic and echocardiographic indices in the abcence of overt clinical symptoms or signs after high-dose CY. Thus, subclinical cardiotoxicity is a common finding after high-dose CY (Kupari et al. 1990a, Braverman et al. 1991, Hertenstein et al. 1994, Cardinale et al. 2002) and may last long (Carlson et al. 1994, Pihkala et al. 1994).

2.3.4.1. Electrocardiography

Resting ECG is widely applied to assess high-dose CY associated cardiac toxicity. ECG abnormalities including non-spesific ST-T changes and benign atrial and ventricular arrhythmias have been observed in about 25 % of patients during or immediately after high-dose CY (Kupari et al. 1990a, Braverman et al. 1991, Murdych et al. 2001). Most often these ECG changes occurred within 1 to 3 days after high-dose CY administration were reversible and returned to baseline within few weeks.

QRS amplitude. Comparisons of the QRS voltages, measured as summations of the R- and S-waves in the limb leads have been used to diagnose or predict cardiotoxicity. The damage to the endothelium and interstitial transudation may result in decreased electrical activity and decreased QRS complex (Gottdier et al. 1981). Among patients with significant CHF, loss of QRS-voltages has been observed in at least 50 to 90 % of the cases treated with high-dose CY (Murdych et al. 2001). However, the decrease in QRS-voltages is generally a late phenomenon, is not always present, and may even coincide with the onset of clinical cardiotoxicity (Cazin et al. 1986, Hertenstein et al. 1994). In addition, decrease in the QRS-voltages has been observed in patients who have developed pleural or pericardial effusion as a result of tumour progression without any chemotherapy related cardiotoxicity (Gottdiener et al. 1981).

QT interval. QT interval prolongation associated with high-dose CY has been reported by Kupari et al. (1990a). In this study one patient experienced ventricular tachyarrhythmias in association with marked QT interval prolongation. Akahori et al. (2003) showed that prolongation in corrected QT interval was more powerful predictor to CHF after high-dose CY than 2D echo parameters. QT dispersion (difference between the maximum and minimum QT-intervals on 12-lead ECG) reflects local or multifocal differences in the repolarization of the myocardium (Day et al. 1990). Increased QT dispersion has been shown in patients with long QT-syndrome, after myocardial infarction and in patients with hypertrophic cardiomyopathy (Day et al. 1990, Buja et al.1993) and has been associated with increased risk for ventricular tachyarrhythmias (Perkiömäki et al. 2002). In addition, increased QT dispersion has been reported to predict acute heart failure following HDT,

particularly high-dose CY containing regimens more effectively than 2D echo parameters (Auner et al. 2002). Nakamae et al. (2000) reported that QT dispersion was an independent predictor of CHF after high-dose CY.

Arrhythmias. Supraventricular arrhythmias and ventricular extrasystolia have been most commonly observed related to high-dose CY (Kupari et al. 1990a, Ando et al. 2000). Hidalgo et al. (2004) reported that especially among older NHL patients supraventricular tachyarrhythmias may predict increased mortality and prolonged hospital stay. In addition, Ando et al. (2000) described a transient complete atrioventricular block in four patients.

2.3.4.2. Heart rate variability

Heart rate variability (HRV), a marker of autonomic nervous regulation, has been shown to be impaired in chronic states like after myocardial infarction and in patients with LV dysfunction (Kleiger et al. 1987, Bigger et al. 1989, Hartikainen et al. 1996). In these situations HRV has been shown to represent an independent marker of cardiac morbidity and mortality (Fauchier et al. 1997). Postma et al. (1996) evaluated HRV in chemotherapy-related cardiotoxicity. Significant impairement in HRV was found in children treated with high-dose CY compared to healthy age-matched subjects. Even though HRV analysis seems to be sensitive test for the detection of high-dose CY induced cardiotoxicity, further studies are needed to clarify its specificity and predictive value.

2.3.4.3. Echocardiography

2D echo provides an accurate measurement of cardiac volumes and systolic function independently of LV shape and regional wall motion disturbances. The most commonly used echo indices of LV systolic function are LVEF and fractional shortening (FS) (Sahn et al. 1978). Echo provides both qualitative and quantitative measures of systolic function. M-mode echo allows measurement of ventricular internal dimensions and wall tickness throughout the cardiac cycle (Otto 2002). The major advantage of M-mode echo is high time resolution, which facilitates recognition of the endocardial borders. Both global and regional ventricular function can be evaluated with 2D echo on a semiquantitative manner.

Assessment of LV size, regional wall motion, and LVEF is readily obtained with 2D echo and is commonly used to distinguish patients with systolic or diastolic cardiac dysfunction. In addition to LV function, end-systolic and end-diastolic volumes can be determined.

Myocardial diseases that result in diastolic dysfunction universally affect both relaxation and chamber stiffness. They result in the need to recruit higher left atrial (LA) pressure to maintain filling at rest or during exertion. An effective atrial contraction is not only necessary to boost LV filling during exercise, but in patients with diastolic dysfunction it becomes an essential compensatory mechanism to enhance LV filling and maintain normal mean LA pressures (Otto 2002).

With the advent of Doppler echo, recording of the transmitral velocity from the apical window using pulse-wave Doppler provides a simple non-invasive approach to the evaluation of diastolic function. The early (E) transmitral velocity recorded at the tips of the mitral valve and its deceleration time, the atrial (A) velocity, and the E/A ratio have become popular indices of diastolic function. The E/A ratio is a normalised index that reflects early diastolic filling relative to atrial contraction (Nishimura and Tajik 1997). The transmitral velocity is also altered by increases in ventricular chamber stiffness and LA pressure. Furthermore, isovolumic relaxation time is the time interval between the closure of the aortic valve and the opening of the mitral valve (Quinones 2005). The transmitral and pulmonal vein velocity are limited in the absence of sinus rhythm and can not be used to assess diastolic function in the presence of mitral inflow obstructions. Because E velocity reflects the interaction between active relaxation and LA pressure, correcting E for the influence of relaxation, should provide an index of LA pressure. In addition, 2D echo allows recognition of concentric LV hypertrophy or remodelling and assessment of LA enlargement, an extremely frequent finding in diastolic heart failure (Otto 2002).

Interestingly, there are two new 2D echo techniques that allow the assessment of myocardial relaxation: propagation velocity by color M-mode and myocardial or annular velocities by tissue Doppler. These indices are less influenced by preload and they can detect early abnormalities of myocardial function (Quinones 2005).

There are several studies where cardiac systolic function during or after high-dose CY has been evaluated by 2D echo. Impairement in LVEF has been a common finding mostly without clinical symptoms (Kupari et al 1990b, Braverman et al 1991, Hertenstein et al. 1994, Murdych et al. 2001, Auner et al. 2002). In addition, 2D echo has revealed a dose-dependent increase in LV mass index and a decrease in FS, resulting in acute reversible decrease in systolic function in almost half of the patients treated with high-dose CY (Kupari et al. 1990b, Braverman et al. 1991). Pericardial effusion has been observed by 2D echo in almost 20 % of patients (Gottdiener et al. 1981, Ando et al. 2000).

Thus, there has been interest in measuring also diastolic parameters to assess cardiac toxicity related to chemotherapy (Benvenuto et al. 2003). In fact, administration of high-dose CY has shown to result in a significant but transient E/A ratio changes without significant change in systolic function suggesting that reversible reduction of LV diastolic compliance may represent the initial sign of cardiac dysfunction (Morandi et al. 2001, Auner et al. 2002).

2.3.4.4. Radionuclide ventriculography

Several studies have assessed cardiac systolic function with serial LVEF measurements by RVG during or shortly after high-dose CY (Bearman et al. 1990, Hertenstein et al. 1994, Brockstein et al. 2000, Lehmann et al. 2000). These studies have reported a slight impairement in LVEF without predictive value for the development of permanent cardiac toxicity. However, LVEF estimation by stress RVG prior to ASCT has shown to have predictive value for peritransplant mortality (Zangari et al. 1999). In this retrospective study, there were several types of malignancies and HDT regimens. One prospective study focused on late cardiac effects of high-dose CY and RVG was performed one, three and five years after treatment. This study concluded that impaired LVEF was a common finding also late after ASCT with only slight or minimal clinical relevance (Carlson et al. 1994).

In addition to measurement of LV systolic function (Wackers et al. 1979) RVG provides the possibility to assess cardiac diastolic function by calculating the peak slope of cardiac filling curve, termed the peak filling rate (PFR) or time to peak filling (TTPF) (Lenihan et

al. 1997). Peak filling occurs during the early active relaxation phase of diastole. TTPF is a measure of this relaxation phase. Although these indices represent a noninvasive assessment of diastolic function, they do not provide any estimate of filling pressure or LV stiffness, and are limited by their sensitivity to loading conditions. Reductions in PFR have been observed in hypertensive patients with LV hypertrophy and in patients with significant coronary artery disease (Bonow et al. 1981). Miller et al. (1986) showed that TTPF varies slightly with heart rate (HR) and does not significantly correlate with age, but that PFR showed significant correlation with both of these measures. However, there is no consensus as to which of these indices should be preferred.

Niwa et al. (2001) showed significant PFR decrease and TTPF prolongation during and shortly after high-dose CY. In another study stress RVG was performed with almost identical findings concluding that impaired diastolic function might provide evidence of cardiotoxicity after high-dose CY (Lele et al. 1996). However, neither study offered any long-term follow-up. Stress RVG study is, however, laborious and not feasible for all patients and therefore seldomly used in clinical practise.

2.3.4.5. Positron emission tomography

Positron emission tomography (PET) is a nuclear imaging technique which is capable of assessing the perfusion and metabolic activity of the myocardium (Ritchie et al. 1995). PET has been shown to effectively distinguish between idiopathic and ischemic cardiomyopathy (Brunken and Schelbert 1989). Gardner et al. (1993a) used PET predicting cardiac toxicity related to high-dose CY. However, in this small study PET possessed no predictive value.

2.3.4.6. Magnetic resonance imaging

Rapid progress has been made in the field of magnetic resonance imaging (MRI) over the past decade. MRI is non-invasive and easy to perform, has high spatial resolution, and avoids the use of potentially nephrotoxic contrast agents or radiation. MRI is based on a tomographic technique that aquires images in virtually any orientation. These images are derived from signals produced by protons. The proton behaves like a small magnet when

placed in a magnetic field and will align parallel and antiparallel to the direction of the primary field, with a small excess of parallel protons that give raise to a net magnetisation vector. Furthermore, this vector can be altered by application of a temporary radiofrequency pulse. A pulse sequence consists of series of radiofrequency pulses with varing duration or strength. Basic pulse sequences used in MRI are spin-echo and gradient-echo sequences, or their faster hybrids. Spin-echo sequences are used for assessment of morphology and the later for ventricular function, wall motions, great vessels and valvular lesions (Salton et al. 2002).

MRI has been established as a reliable and reproducible technique for the assessment of cardiac structure, function and perfusion (Constantine et al. 2004). In addition, MRI is a precise method for measurement of right ventricular and LV systolic and diastolic functions and mass (Bellenger et al. 2000). Quantification of LV volumes and function is possible to perform with geometric models like the modified Simpson's rule or with a three-dimensional (3D) data set. There are no studies using MRI to assess high-dose CY related cardiac toxicity.

2.3.4.7. Cardiac biomarkers

Extensive and expensive monitoring programs are recommended to identify patients developing cardiac toxicity related to chemotherapy (Ritchie et al. 1995). Echo has shown rather poor predictive value in studies dealing with anthracycline cardiotoxicity (Lipshultz et al. 1993). In recent years the use of biochemical markers in addition to imaging methods has been studied in association to high-dose CY.

Troponins. Cardiac troponins (cTnT, cTnI) are spesific biochemical markers of myocardial damage which are not normally present in serum or plasma. These thin-filament contractile proteins are present in high concentrations in the myocardium and are released into the circulation after myocyte injury. Both troponins are released within 4-12 h following an episode of myocardial necrosis with a peak value 12 to 48 h after the injury (Adams et al 1993, Penttilä et al. 1999).

Cardinale et al. (2000) reported a study, in which plasma cTnI levels and LVEF was assessed in patients receiving high-dose CY. Patients with increased cTnI had a greater impairment in LVEF. In another study the same investigators divided patients into different risk groups for cardiac toxicity according to elevations in cTnI soon after the high-dose CY (Cardinale et al. 2004). According to two other studies on single-agent high-dose CY, serial plasma measurements of cTnI and cTnT were uniformly negative (Morandi et al. 2001, Auner et al. 2002). Despite these conflicting data, the lack of troponin elevation following high-dose CY might suggest the absence of direct membrane damage of myocytes.

Natriuretic peptides. The prohormones of A- and B-type natriuretic peptides are produced by cardiac myocytes and cleaved on secretion into biologically active peptides (ANP and BNP) and their N-terminal counterparts (NT-proANP and NT-proBNP). Elevated levels of natriuretic peptides are associated with increased atrial wall stretch (due to volume expansion and pressure over load) or ventricular wall stretch (due to reduced ventricular systolic and diastolic function) (Ruskoaho 2003). It has been suggested, though, that the primary stimulus for ANP and BNP secretion is exerted by atrial distension. These cardiac hormones protect the heart from adverse consequences of overload by increasing natriuresis and diuresis, relaxing vascular smooth muscle, inhibiting the renin-angiotensin-aldosterone system, and by counteracting cardiac hypertrophy and fibrosis. Because natriuretic peptides are regulators of salt and fluid homeostasis, they are therefore indicators of cardiac homeostatic response and dysfunction (Magga et al. 1998). ANP is also supposed to be an endogenous regulator of the inflammatory response (Vollmar 2005).

In patients with CHF natriuretic peptide values are elevated, because the cardiac hormonal system is activated by increased wall stretch due to volume and pressure overload. In addition, these patients have elevated circulating tissue levels of norepinephrine, angiotensin II and endothelin-1. These are all cytokines that can not only increase the hemodynamic stress on the ventricle, but also directly stimulate ANP expression and release from cardiac myocytes. In patients with severe CHF, the concentrations of BNP increase 10-50-fold higher than ANP (Pandey 2005). These findings indicate that ANP and BNP elicit distinct physiological and pathophysiological effects.

Although the plasma concentrations of BNP are significantly increased in heart failure, they are insufficient to produce the biological effects of natriuretic peptides, suggesting that severe heart failure is a state of relative deficiency of natriuretic peptides (Chen and Burnett 2000). Recently it has been also reported that natriuretic peptides predict short-term mortality in patients with chronic CHF (Stanton et al. 2005).

NT-proBNP has been proposed as a tool for detecting asymptomatic LV dysfunction (Maisel et al. 2001). On the other hand, NT-proANP has also been able to differentiate between asymptomatic heart failure patients and healthy subjects (Lerman et al. 1993). Both NT-proANP and NT-proBNP values have been higher in patients with diastolic dysfunction and preserved systolic function compared to healthy subjects (Krishnaswamy et al. 2001).

Patients with septic shock show reversible LV systolic dysfunction commonly masked by a concomitant elevation in the cardiac index. Cytokines and endotoxins from Gram-negative micro-organisms may lead to myocardial depression and ventricular dilatation (Parillo 1993). The cardiovascular response to septic shock is peripheral vasodilatation resulting in systemic hypotension, hyporesponsiveness to vasopressors and reduced systemic vascular resistance. Furthermore, NT-proANP and NT-proBNP may serve as useful biomarkers to indicate myocardial dysfunction and may help to differentiate between survivors and non-survivors of severe sepsis (Brueckmann et al. 2005).

Natriuretic peptide measurements have been used to monitor cardiotoxicity after HDT. Until now three studies have been published in association to high-dose CY containing therapy. In the study by Snowden et al. (2000) patients who developed clinical heart failure exhibited slightly elevated BNP levels in serial measurements before the onset of symptoms. Niwa et al. (2001) reported an elevation on natriuretic peptides very shortly after ASCT (day +1 and +14), but neither peak was predictive of cardiac toxicity. In the most recent study persistently increased NT-proBNP just after high-dose CY was strongly asssociated with development of subclinical cardiac dysfunction (Sandri et al. 2005).

Additional factors that can affect natriuretic peptide values are presented in Table 6. BNP is increased in late stage of renal failure, which is in part related to the decreased renal

clearance and accompaning increased intravascular volume (Bettencourt 2005). Furthermore, BNP is increased in early course of acute myocardial infarction due to systolic dysfunction, impairment of ventricular relaxation and stunning of the myocardium. A second peak of BNP measured 2-4 days after myocardial infarction is associated with remodelling of the heart (Sagnella 1998). Myocardial ischemia can elicit the release of BNP in the absence of necrosis. The association with female gender and BNP appears to be related to estrogen status (Redfield et al. 2002). Furthermore, BNP increases also with age, presumably as a result of LV stiffness and progressive deterioration of renal function. Because adrenergic stimulation inhibits the release of BNP, initiation of β-blockers might slightly increase natriuretic peptide concentrations (Bettencourt 2005).

Diuretics, vasodilatators, ACE-inhibitors, spironolactone and angiotensin II receptor antagonists lead to decreased BNP values (Sagnella 1998). The observation of lower BNP concentrations in obese people remains unexplained. Furthermore, age, gender and assay-spesific values are needed.

Table 6. Factors that might increase or decrease natriuretic peptide values (Ruskoaho 2003, Bettencourt 2005).

Increase	Decrease
Age	Obesity
Female gender	Cardiovascular medication
Renal failure	ACE-inhibitors
Myocardial infarction	Diuretics
Acute coronary syndrome	β-blockers
Lung disease with right-sided failure	Spironolactone
Acute, large pulmonary embolism	
Tachycardia	
Valvular diseases	
Ventricular hypertrophy	
Cardiomyopathy	
Glucocorticoid use	

Abbreviations: ACE, angiotensin-converting enzyme

2.3.5. Risk factors and prevention of cardiac toxicity

Some authors have concluded that older age represents a risk factor for the development of cardiovascular complications after ASCT (Brockstein et al. 2000) but limited data are available on this issue. TBI as part of HDT does not seem to increase the risk for cardiac toxicity (Carlson et al. 1994, Auner et al. 2002). Futhermore, prior radiation therapy to the mediastinum or left chest wall seems to represent a risk factor (Ikäheimo et al. 1985, Adams et al. 2003). In overweight patients the high-dose CY dosage adjustment is important to prevent cardiac toxicity (Morandi et al. 2001).

2.3.5.1. Administration and dosage of high-dose CY

It has been suggested that cardiac toxicity can be avoided if high-dose CY is administrated at a dose/m^2 rather than per kilogram (Goldberg et al. 1986). In recent years, the percentage of patients receiving single-agent high-dose CY up to 7 g/m^2 experiencing cardiotoxicity has diminished with the adoption of multifractioned schedule of administration (Hertenstein et al. 1994, Morandi et al. 2005).

2.3.5.2. Concomitant administration of other chemotherapeutic agents

Several widely employed combinations of high-dose CY have not been associated with an increased risk of cardiotoxicity over single-agent high-dose CY (Petros et al. 2002). Pericarditis and arrhythmias have been raported after high-dose cytarabine (1-3 g/m^2) (Vaickus and Letendre 1984). When high-dose CY and cytarabine were coadministered the highest incidence of cardiac toxicity was reported (Appelbaum et al. 1976, Gottdiener et al. 1981, Cazin et al. 1986). Significant cardiotoxicity has been reported using high-dose mitoxantrone with high-dose CY. In that study four out of six patients without pre-existing cardiac disease experienced severe cardiac toxicity and two cardiac deaths were reported (Gralow et al. 2001). Even though high-dose melphalan has rarely been associated with cardiac dysfunction (Spriano et al. 1994), there are two reports in which this drug was used in high-doses as a combination with 2-14 % incidence of cardiac toxicity (Giralt et al. 2001). Melphalan is, however, seldom used in combination with high-dose CY.

2.3.5.3. Previous anthracycline therapy

The assumption that the incidence of chronic cardiomyopathy related to anthracyclines can be minimised by restricted the cumulative anthracycline dose to 400 mg/m^2 has been challenged (Meinardi et al. 2002). Sakata-Yanimoto et al. (2004) showed that cumulative anthracycline dose was a predictor of cardiac complications and they recommended avoiding anthracyclines within two months before ASCT. However, some studies have not confirmed a relationship between previous anthracycline exposure and development of cardiac toxicity following high-dose CY (Cazin et al. 1986, Braverman et al. 1991, Morandi et al. 2001, Fujimaki et al. 2001).

High-dose CY associated cardiac damage was the most frequent in pediatric patients receiving CY > 5 g/m^2 or in those receiving CY > 2 g/m^2 who had also received > 100 mg/m^2 anthracyclines prior to transplant (Steinherz et al. 1981). Also patients treated with a cumulative anthracycline dose below 400 mg/m^2 may experience subclinical or even clinical toxicity during or many years after therapy (Kremer et al. 2002). Therefore, there is no safe anthracycline cumulative dose prior to high-dose CY that could be recommended.

2.3.5.4. Cardiac systolic function prior to ASCT

Preserved cardiac function is generally required for the enrollment of patients into HDT protocols. This is defined as LVEF > 50 % and absence of other significant cardiac disease. However, measurement of LVEF before HDT is of limited practical value and has been shown to be unable to predict future cardiac toxicity (Zangari et al. 1999, Brockstein et al. 2000, Lehmann et al. 2000). One study reported that increased risk of milder cardiac events rather than increased mortality due to severe cardiac toxicity was found among patients with impaired baseline LVEF. In fact, two thirds of major cardiac events occured in patients with normal LVEF (Hertenstein et al. 1994). On the other hand, there are studies showing increased incidence of severe cardiac complications in patients with low LVEF prior to ASCT (Bearman et al. 1990, Braverman et al. 1991, Fujimaki et al. 2001). Overall, resting LVEF measurement in every patient proceeding to HDT is not recommended (Bearman et al. 1990, Hertenstein et al. 1994).

Since the predictive value of cardiologic evaluation is limited, exclusion of patients from HDT protocols based on slightly impaired cardiac function should be reconsidered (Hertenstein et al. 1994, Rose et al. 2000). A detailed patient history, physical examination, chest X-ray and ECG are important tools for detecting candidates at high risk for cardiac complications (Bearman et al. 1990, Murdych et al. 2001). Cardiac function evaluation is recommended prior to HDT for patients with history, symptoms or signs of cardiac disease, history of prolonged anthracycline exposure or prior mediastinal radiotherapy.

2.3.6. Management of cardiac toxicity

Treatment of high-dose CY associated clinical heart failure does not differ from the general approach in patients with heart failure. Diuretics and angiotensin-converting enzyme (ACE) inhibitors in case of impaired LV function should be considered according to established quidelines (Swedberg et al. 2005). Digoxin therapy might also be considered if heart failure persists. Particularly in patients with acute heart failure sustained or recurrent cardiac arrhythmias should be treated with appropriate antiarrhythmics. ACE-inhibitor (enalapril) has been started in profylactic treatment prior to HDT to prevent deterioration of pre-existing subclinical LV dysfunction (Kakavas et al. 1995). In this small study including only six patients, LVEF improved during HDT. There is need for non-invasive and cost-effective approaches to identify cardiac toxicity of HDT already at the preclinical stage. This would allow clinicians to more closely monitor cardiovascular function, to prevent cardiac function impairment and to start treatment in early phase.

3. AIMS OF THE STUDY

In this study, several aspects of high-dose therapy supported by autologous stem cell transplantation in NHL patients were studied. The specific aims were:

To identify pre-and postmobilisation factors predicting failure in haematopoietic progenitor cell mobilisation **(Study I)**

To evaluate the value of chemotherapy scoring to predict haematopoietic progenitor cell mobilisation **(Study II)**

To compare the toxicity and efficacy of BEAC and BEAM regimens **(Study III)**

To assess the very acute cardiac effects of BEAC regimen **(Study IV)**

To investigate cardiac effects within three months after BEAC regimen **(Study V)**

To identify prognostic factors for treatment response and survival in patients with progressive disease after ASCT **(Study VI)**

4. PATIENTS AND METHODS

4.1. Patients and chemotherapy

4.1.1. Prediction of haematopoietic progenitor cell mobilisation (Studies I-II)

Altogether 97 (Study I) and 120 (Study II) adult NHL patients underwent HPC mobilisation with intermediate-dose CY 4 g/m^2 followed by G-CSF and progenitor cell aphaeresis. In Study I, 59 patients were males and 38 females, with a median age of 49 years (16-70). DLBCL was the most common histology (50 patients, 52 %). BM involvement was found in 17 patients (18 %). The median time from dignosis to HPC mobilisation was 7 months (3-120).

In study II, 70 patients (61 %) were males and 50 (39 %) were females. The median age was 49 years (16-70) and 62 patients (52 %) had DLBCL. The median number of prior chemotherapy regimens was 2 (1-5) and prior chemotherapy cycles 8 (3-29).

CY 4 g/m^2 was infused during 90 minutes. Mesna (sodium-2-mercapto-ethane sulphonate) 1600 mg/m^2 was given intravenously 30 minutes before starting the CY infusion and 3 and 6 hours after the CY infusion. Furthermore, G-CSF was started 48 hours after the CY infusion. G-CSF was continued until the end of aphaeresis or until failure of HPC mobilisation was evident.

4.1.2. Toxicity and efficacy of BEAC and BEAM regimens (Study III)

Altogether 71 NHL patients receiving HDT supported by ASCT were analysed. The patients had either relapsed NHL or were treated with HDT in first remission based on initial poor-risk features (IPI) or failure to achieve remission with standard chemotherapy. The HDT regimens applied were BEAC (N=36) or BEAM (N=35). During the study period, two variants of BEAM were used.

BEAC consisted of BCNU 300 mg/m^2, etoposide 800 mg/m^2, cytosine arabinoside 800 mg/m^2 and CY 140 mg/kg. BEAM1 (N=11) included BCNU 300 mg/m^2, etoposide 800 mg/m^2, cytosine arabinoside 800 mg/m^2 and melphalan 140 mg/m^2. BEAM2 regimen (N=24) consisted of BCNU 300 mg/m^2, etoposide 1200 mg/m^2, cytosine arabinoside

1600 mg/m^2 and melphalan 140 mg/m^2. Prophylaxis against *Pneumocystis carinii* consisted of cotrimoxazole for four months. In addition, mouth care was recommended to perform with miconatsole 2 % oral gel twice a day. No other prophylactic measures were applied. All patients received G-CSF 5μg/kg/d s.c. after the progenitor cell infusion until neutrophils were > 1.0 x 10^9/l.

In BEAC group, the median age was 46 years (27-66) and male dominance was observed (23 patients, 66 %). Moreover, 15 patients (42 %) had DLBCL and the median IPI was 2 (0-4). In BEAM group, the median age was 48.5 years (16-70) and 20 of patients (70 %) were males. Altogether 18 patients (50 %) had DLBCL and median IPI was 2 (0-4).

4.1.3. Cardiac effects of BEAC regimen (Studies IV-V)

In study IV, seventeen adult NHL patients with a median age 56 years (25-66) who were scheduled to receive HDT, were studied. These patients also participated Study V simultaneously. Altogether 15 patients (88 %) were males and DLBCL was the most frequent histology (eight patients, 47 %). Three patients had pre-existing cardiovascular disease (WHO class II hypertension without left ventricular hypertrophy). They were treated with angiotensin-converting enzyme inhibitors or calcium channel blockers, but no diuretics or β-blockers were used. One patient had type 2 diabetes mellitus without complications. All patients had earlier received anthracyclines with a median cumulative dose of 400 mg/m^2 (150-500). Mediastinal radiotherapy had been previously administrated to one patient.

In study V, 30 adult NHL patients scheduled to receive BEAC supported by ASCT, were studied. The median age of the patients was 56 years (25-66). Twenty-four were males (80 %) and DLBCL was the most common histology (18 patients, 60 %). All patients had received anthracyclines with a median cumulative dose 400 mg/m^2 (150-650). Furthermore, two patients suffered from type 2 diabetes mellitus without complications. Altogether nine patients (30 %) had pre-existing cardiovascular disease: WHO class II hypertension in seven patients and Canadian Cardiovascular Society class 1 coronary heart disease with previous myocardial infarction in two patients. These patients were treated

with angiotensin-converting enzyme inhibitors (N=5), calcium channel blockers (N=2), thiazide diuretics (N=2) and β-blockers (N=2).

In studies IV and V, HPC mobilisation was performed with CY 4g/m^2 + G-CSF in all patients without cardiac complications. BEAC chemotherapy was administrated in doses (carmustine 300 mg/m^2 d-7, sytarabine 200 mg/m^2/d, etoposide 200 mg/m^2/d and cyclophosphamide 1500 mg /m^2/d plus Mesna 600 mg/m^2/d from d-6 to d-3) via central venous catheter followed by stem cell infusion on day=0 (D0). Cyclophosphamide was given as a 1 h infusion during four days with a total dose of 6000 mg/m^2 and hyperhydration (≥ 4 liters/day) was used during BEAC. Furthermore, none of the patients received mediastinal radiotherapy or further chemotherapy within three months after ASCT.

Between d-7 and D0, none of the patients developed fever or infections. During the study cardiac failure was diagnosed based on clinical symptoms (fluid retention, dyspnoea, ortopnoea) and findings (peripheral oedema, increased jugular venous pressure, sinus tachycardia) and supported by chest X-ray findings. In addition, after d+2 all patients experienced neutropenic fever (Study V). The concentration of DMSO was 10 % in aphaeresis product.

4.1.4. Progressive disease after ASCT (Study VI)

Altogether 353 adult NHL patients received HDT supported by ASCT 1991-2000 in six Finnish transplant centres. Until April 2001 progressive disease was observed in 115 patients (33 %). These patients were included in the study. Seventy six patients (66 %) were males and the median age was 49 years (16-70) at the time of HDT. The histology included DLBCL in 52 patients (45 %), FL in 26 patients (23 %), MCL in 15 patients (13 %) and T-NHL in 16 patients (14 %). Over half of the patients (53 %) had received HDT as second-line salvage therapy.

4.2. Methods

4.2.1. Factors predicting haematopoietic progenitor cell mobilisation (Studies I-II)

All patient records including pathological, radiological and laboratory data were evaluated by a single observer. Premobilisation factors including age, gender, lymphoma subtype, AA-stage, time from diagnosis to mobilisation, previous chemotherapy and BM involvement were noticed. In addition, several postmobilisation factors including neutropaenic fever, need for supportive care, blood count nadirs and peak blood $CD34^+$ (B-$CD34^+$) counts were taken into account for each patient. The day of CY infusion was defined as D0.

Aphaeresis was started routinely if the morning B-$CD34^+$ count was > 20 x 10^6/l. However, aphaeresis was begun in 30 patients (31 %) with B-$CD34^+$ cell counts between 5-20 x 10^6/l with raising WBC counts. The aim was to collect > 2 x 10^6/kg $CD34^+$ cells (> 5 x 10^6/kg if $CD34^+$ selection was intended). The minimum criterion for successful mobilisation was the collection of at least 1.5 x 10^6/kg $CD34^+$ cells after a single mobilisation. All other outcomes were regarded as mobilisation failures (Study I).

The criterion used for excellent mobilisation was the collection of > 5 x 10^6/kg $CD34^+$ cells after a single mobilisation or > 2 x 10^6/kg with a single apheresis. The mobilisation was regarded as sufficient when at least 1.5 x 10^6/kg $CD34^+$ cells were collected following a single mobilisation, but not fulfilling the criteria for excellent mobilisation. The mobilisation failure was defined as no apheresis performed due to low B-$CD34^+$ counts or collection of < 1.5 x 10^6/kg $CD34^+$ cells after a single mobilisation attempt (Study II).

To study the quality and quantity of previous chemotherapy on the efficiency of HPC mobilisation, the original scoring system by Drake et al. (1997) was used. Since several widely applied chemotherapy regimens were missing from that scoring, an improved chemotherapy scoring was developed (Table 7) based on pharmagolocical data and clinical experience of these agents.

Table 7. The improved chemotherapy scoring for NHL patients. Only changes to the original scoring system by Drake et al. (1997) are shown.

Toxicity factor	Drug
0	Methylprednisolone
1	Methotrexate, cytosine arabinoside
2	Ifosfamide, mitoguazon, mitoxantrone
3	Fludarabine, dacarbazine
4	-

The chemotherapy score for each cycle was calculated by summing the toxicity factors of individual drugs and multiplying this sum by the number of chemotherapy cycles given before progenitor cell mobilisation (e.g. CHOP=2+2+1+0=5 points/cycle; CHOP 8 cycles=40 points; DHAP=0+1+2=3 points/cycle).

All aphaereses were performed with a Cobe Spectra^{R} cell separator (Cobe Laboratories Ltd., Gloucester, UK). Aphaereses (10-15 l) were performed lasting 3-4 hours and the number of $CD34^{+}$ cells was counted after each aphaeresis. Central venous catheter (Vascath^{R}) was used for HPC aphaeresis in more than 90 % of the patients.

4.2.2. Toxicity and efficacy of BEAC and BEAM regimens (Study III)

The data was prosplectively collected from a single hospital ASCT database. All patient charts including laboratory files and daily nursing reports were screened for completion of data. The assessment of oral mucositis and diarrhoea was based on daily nursing reports and clinical examination by a treating physician. The criterion for engraftment was neutrophils $> 0.5 \times 10^{9}$/l and platelets $> 20 \times 10^{9}$/l. For assessment of toxicity, a WHO grading for acute and subacute cancer treatment toxicity was used (Miller et al. 1981) (Table 8). Only toxicity of grade > 2 was taken into account.

Table 8. WHO criteria for evaluation of acute and subacute toxicity in cancer therapy (Miller et al. 1981). Only grading for gastrointestinal, infectious, renal, pulmonary and cardiac toxicity is presented.

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Gastrointestinal					
oral	none	soreness/ erythema	eryhtema, ulcers can eat solids	ulcers, liquid diet	alimentation not possible
diarrhoea	none	transient < 2 days	tolerable, > 2 days	intolerable, requiring therapy	hemorrhagic dehydration
bilirubin	< 1.25xN	1.26-2.5 x N	2.6-5 x N	5.1-10 x N	>10 x N
alkaline phosphatase	< 1.25 x N	1.26-2.5 x N	2.6-5 x N	5.1-10 x N	>10 x N
Infections	none	minor infection	moderate infection	major infection	major infection with hypotension
Cardiac					
rhythm	none	sinus tachycardia > 110 at rest	unifocal PVC atrial arrhythmia	multifocal PVC	ventricular tachycardia
function	none	asymptomatic but abnormal cardiac sign	transient; no therapy	symptomatic, responsive to therapy,dys-function	symptomatic, non-response to therapy, dysfunction
pericarditis	none	asymptomatic effusion	symptomatic no tap	tamponade tap required	tamponade,surgery required
Pulmonary	none	mild symptoms	exertional dyspnea	dyspnea at rest	complete bed rest required
Fever	none	fever 38	fever 38-40	fever > 40	fever with hypotension
Renal, bladder					
proteinuria	none	< 0.3g/ 100 ml	0.3-1.0g/ 100 ml	> 1.0g/ 100 ml	nephrotic syndrome
hematuria	none	microscopic	gross	gross + clots	obstructive uropathy
creatinine	<1.25 x N	1.26-2.5 x N	2.6-5 x N	5-10 x N	>10 x N

Abbreviations: PCV, premature ventricular contraction; N, upper limit of normal

4.2.3. Cardiac evaluation of BEAC regimen (Studies IV-V)

The study protocol for prospective studies evaluating cardiac effects of BEAC regimen was as follows:

	d-7	d-6	d-3	d-2	D0	d+7	d+12	m+3
BEAC [#]		X----------		X				
Stem cell infusion					X			
RVG	X						X	X
Natriuretic peptides	X			X		X	X	X
MRI[##]	X			X				

Abbreviations: CY, cyclophosphamide; d, day; m, month; RVG, radionuclide ventriculography; MRI, magnetic resonance imaging [#]high-dose CY with total dose 6000 mg/m^2 as part of BEAC regimen [##]MRI was performed for 17 patients (Study IV).

In addition, body weight and temperature, blood pressure and HR were measured on each morning during the study.

4.2.3.1. Magnetic resonance imaging (Study IV)

The first MRI study was performed at baseline on d-7 just prior to HDT and the second evaluation was on d-2 just after completing BEAC regimen. MRI was performed with a 1.5 T clinical MR scanner (Magnetom Vision^R, Siemens Medical Systems Inc., Erlangen, Germany) at 7 a.m. A phased array body coil was used as a receiver. Transaxial localizer images were acquired to define LV and LA. After that, cine images were obtained using an ECG-gated gradient echo sequence (TR 50 ms, TE 4.8 ms). First cine imaging level was an oblique sagittal long axis image through LV apex through mitral valve annulus and LA that was positioned using transaxial scout images. Next imaging plane was the long axis cine image through LV apex through mitral valve annulus and LA that was set perpendicularly to the previous oblique sagittal end-diastolic cine image.

LV length was measured in the horizontal long-axis plane. This length was devided into three equal sections. Thereafter, two short axis cine images were obtained perpendicularly to the both long axes of the left ventricle at the first and second third of the LV length. Cross-sectional area of the LV myocardium was measured in short axis images at the first and second third of the LV length in end-diastolic and end-systolic images. Left ventricular end-systolic (ESV) and end-diastolic (EDV) volumes were calculated using the modified Simpson's rule (Dulce et al. 1993). LVEF was calculated using an equation LVEF = ((EDV-ESV)/EDV) x 100 % and LV mass = LV volume x 1.05 (Katz et al. 1988). Furthermore, stroke volume (SV) was calculated using an equation SV= EDV-ESV and cardiac output (CO) = SV x HR. A LVEF decrease $\geq$ 10 % units from the baseline or LVEF < 50 % were defined as abnormal.

The technique has previously been validated in the study centre by comparison of LVEF values (by geometrical modified Simpson's rule) in healthy subjects and it has a good inter-study reproducibility, 7 % (Sipola, unpublished data). In a small study Dulce (1993) showed no significant differences between values obtained with the modified Simpson's rule versus the 3D data set for LVEF. In addition, a high inter-observer reproducibility of MRI measurements within these two models was found.

4.2.3.2. Radionuclide ventriculography (Study V)

Equilibrium RVG was performed with *in vivo* technetium-99m-labeled blood cells (dose 670 MBq). A large field-of-view gamma camera equipped with a high-sensitivity parallel hole collimator was used for imaging. The cardiac cycle was divided into 32 frames with a 10 % tolerance. Ten million counts were acquired. Data were analysed with commercial cardiac software (MGQ, Nuclear Diagnostics AB, Hägersten, Sweden). Systolic LV function was assessed by measurement of LVEF. LV diastolic function was estimated by TTPF. LVEF < 50 % was defined as systolic dysfunction (Wackers et al. 1979) and TTPF > 180 ms as diastolic dysfunction (Miller et al. 1979). The RVG method used had been validated earlier in the study centre (Nousiainen et al. 1999).

4.2.3.3. Natriuretic peptide measurements (Studies IV-V)

The blood samples were drawn into chilled tubes containing 1.5 mg K2-EDTA/ml blood after the patient had been in the supine position for 30 min at 8 a.m. The whole blood was centrifuged and plasma immediately frozen and stored at -70 C. NT-proANP and NT-proBNP were assayed directly from unextracted plasma. Circulating NT-proBNP (antiserum to NT-proBNP$_{10-29}$) and NT-proANP (antiserum to NT-proANP$_{46-79}$) concentrations were determined by radioimmunoassay as described by Ala-Kopsala et al. (2004). With the NT-proANP$_{46-79}$ assay, the range of serum concentrations in healthy individuals was 81-571 pmol/l. The mean standard deviation (SD) was 255 (95) pmol/l. With the NT-proBNP$_{10-29}$ assay, the range of serum concentrations in healthy individuals was from undetectable to 250 pmol/l. The mean SD was 86 (32) pmol/l. The values in healthy adults are for NT-proANP < 455 pmol/l and NT-proBNP < 150 pmol/l, without an effect of gender or age (Ala-Kopsala et al. 2004).

4.2.4. Progressive disease after ASCT (Stydy VI)

The data was collected from medical charts by using a designed form and by a single investigator (T.K.). The first routine evaluation of treatment response was performed at about three months after HDT. In patients who had achieved a CR after ASCT, a progression was defined as any later evidence of NHL. Also those patients who had achieved a partial remission (PR) after ASCT or were refractory, but later showed new evidence of NHL, were considered to have progressive disease. Also patients in whom evidence of NHL was observed before the first response evaluations were also included into this series. The date of progression was the date of diagnostic specimen or diagnostic study showing evidence of NHL progression.

For the definition of the response to salvage therapy CR was defined as disappearance of all clinical and radiological evidence of NHL. PR was defined as at least 50 % reduction in tumour mass. All other outcomes were considered as non-responders (NR). Response was evaluated based on radiological evaluation (ultrasound, computed tomography), clinical examination, and in a case of disseminated NHL on BM biopsy.

4.3. Statistical analysis

All calculations were performed with the SPSS for Windows Releases 7.0, 9.0, 10.0 or 11.0 (SPSS Inc., Chicago, IL, USA). A P value < 0.05 was considered statistically significant. The statistical significance of a single factor in univariate analyses was evaluated using the Chi-square test for categorical variables and the Mann-Whitney U test for continuous variables (Studies I-VI).

In study I, the significant parameters associated with mobilisation failure in univariate analysis were investigated in multivariate analysis using a logistic regression. To predict the mobilisation failure for each patient, the following equation was used:
Probability (P) = $1/ (1+exp (-Z))$, *where* $Z= b_0+ b_1X_1+ b_2 X_2+...bpXp.$ $X_1, X_2,...X_k$ *are covariates and* $b_0, b_1,.....b_k$ *are their estimated coefficients.* The ROC (receiver operating characteristics) curve of the predictive mathematical model was built by plotting sensitivity against 1-specificity for different probability values.

In study II, Spearman's correlation test was used to investigate the association between the chemotherapy score and the peak blood $CD34^+$ count as well as the number of $CD34^+$ cells collected. ROC curve was used to evaluate the ability of chemotherapy score to predict the outcome of mobilisation.

In study III, Kaplan-Meier method was applied to describe OS and PFS after HDT. Log-rank test was used to analyse possible differences in survival.

In studies IV-V, paired two-tailed t-test was used to compare means and the correlations between variables were studied using Spearman's correlation test. In study V, the subgroup analyses were performed using a general linear method with repeated measures ANOVA. Time (five time points or four change points) was defined as a within-subject factor and LVEF at baseline ($\geq$ or < 50 %) as a between-subject factor. P values for both group x time interaction and time effect were defined by the Greenhouse-Geissler. The data are expressed as mean $\pm$ standard error of mean (SEM).

In study VI, a logistic regression was used defining the predictors to CR or PR status after salvage therapy. Kaplan-Meier survival curves were generated and comparisons made by using the log-rank test. Factors significantly associated with survival were then entered

into a multivariate Cox model. Survival times were calculated from the time of progression after ASCT to death or the date of last follow-up.

4.4. Approval of the Ethics Committee

Written informed consent was obtained from all the patients participating prospective studies (Study IV-V) after the purpose of the study was explained to them. The protocol was approved by the Ethics Committee of the University of Kuopio (136/11/00) and was in accordance with the Helsinki Declaration.

5. RESULTS

5.1. Prediction of mobilisation failure (Study I)

Seventy-nine patients (81 %) were successful mobilisers, whereas eighteen patients (19 %) experienced a mobilisation failure with the definition used. Altogether 29 of those 30 NHL patients, whose aphaeresis was started with B-CD34$^+$ cell counts between 5-20 x 10^6/l, experienced a successful mobilisation and only one patient was a mobilisation failure.

Of the premobilisation factors, BM involvement at the time of diagnosis (P=0.001) or prior to mobilisation (P=0.001) was associated with mobilisation failure in univariate analysis. In addition, a low platelet count just prior to mobilisation also predicted failure (P=0.001), as did a low WBC count (P=0.038) and a low absolute neutrophil count (P=0.035). Further, several postmobilisation factors were associated with mobilisation failure in univariate analyses: a low WBC nadir (P<0.001), low platelet nadir (P<0.001), neutropenic fever (P=0.001) and need for platelet transfusions (P<0.001). A longer time to reach peak B-CD34$^+$ level (P<0.001) and a lower peak B-CD34$^+$ level after mobilisation (P<0.001) were also statistically significant. In multivariate analysis only two premobilisation factors, BM involvement at the time of diagnosis (P=0.004) and platelet count just prior to mobilisation (P=0.01), retained their statistical significance in predicting mobilisation failure.

The area under the ROC curve built on the mathematical model was 82 % ((confidance interval (CI) 95 %; 0.71-0.93)) (Figure 1). The detected threshold value for a continuous variable (platelet count just prior to mobilisation) to predict mobilisation failure was a platelet count 190 x 10^9/l with a sensitivity of 0.71 (CI 95 %; 0.49-0.92), specificity of 0.77 (CI 95 %; 0.68-0.86), a positive predictive value of 0.40 (CI 95 %; 0.22-0.57) and a negative predictive value of 0.92 (CI 95 %; 0.86-0.99). Examples of using the mathematical model based on the results of the multivariate analysis are presented in Table 9.

Table 9. Risk of mobilisation failure according to the mathematical model.

Bone marrow involvement at diagnosis	Platelet count just prior to mobilisation (x 10^9/l)	Risk of mobilisation failure
+	100	61 %
+	150	55 %
+	200	39 %
-	100	19 %
-	150	13 %
-	200	9 %

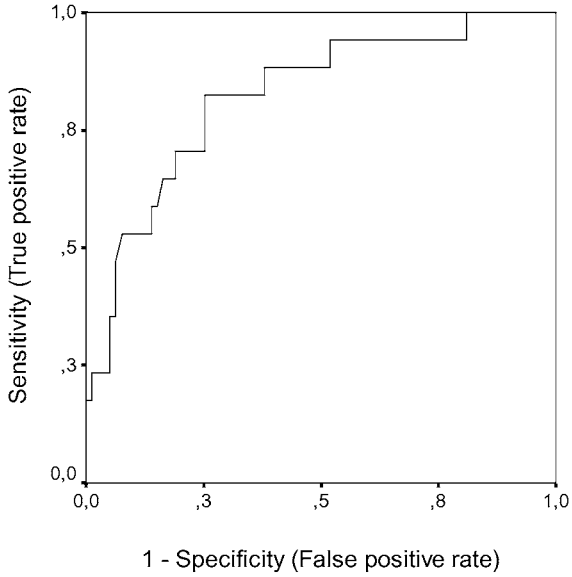

Figure 1. ROC curve showing sensitivity (true-positive rate) and 1-spesicificity (false-positive rate) of the mathematical model to predict the mobilisation failure for each NHL patient. Area under the curve = 0.82; 95 % CI = 0.71-0.93.

5.2. Chemotherapy scoring to predict mobilisation (Study II)

HPC mobilisation was successful in 101 patients (84 %), whereas 19 patients (16 %) experienced a mobilisation failure. Seventeen patients (14 %) had received TF 3 drugs (fludarabine 8 pts, chlorambucil 7 pts, procarbazine 1 pt, and dacarbazine 1 pt) and only two patients (2 %) TF4 drugs (metchlorethamine) prior to HPC mobilisation.

Application of the original chemotherapy scoring by Drake et al. (1997) was possible only in 32 patients (27 %). The main reason was that many commonly used agents were missing from the original scoring system. Out of 32 patients who could be adequately scored, only three patients (9 %) failed to mobilise, whereas nine patients (28 %) fulfilled the criterion for sufficient and 20 patients (63 %) the criterion for excellent mobilisation, respectively. The scores between the patients who failed to mobilise were higher than in those who achieved the minimum target yield after the mobilisation (medians 48 vs. 35), but the difference did not reach statistical significance. No significant differences were observed in the chemotherapy score between those patients who had excellent mobilisation *vs.* those with sufficient mobilisation either. No significant correlation between the score and the peak B-CD34$^+$ counts or the aphaeresis yield was observed in this patient group.

By using the improved scoring system altogether 111 patients out of 120 (93 %) could be scored. Out of these 111 patients, 14 (13 %) failed to mobilise according to the criterion used whereas 63 patients (58 %) had an excellent mobilisation and in additional 34 patients (30 %), a sufficient graft was collected. The median score was 44.5 for patients who failed mobilisation, 46.5 for those with a sufficient mobilisation and 36 for those who showed an excellent mobilisation (P=NS). There was a significant inverse correlation between the chemotherapy score and the peak B-CD34$^+$ count measured (r=-0.214, P=0.024) as well as between the improved chemotherapy score and yield of the aphaeresis (r=-0.234, P=0.02). However, the ROC showed no threshold value for the improved chemotherapy score to predict mobilisation failure (Figure 2).

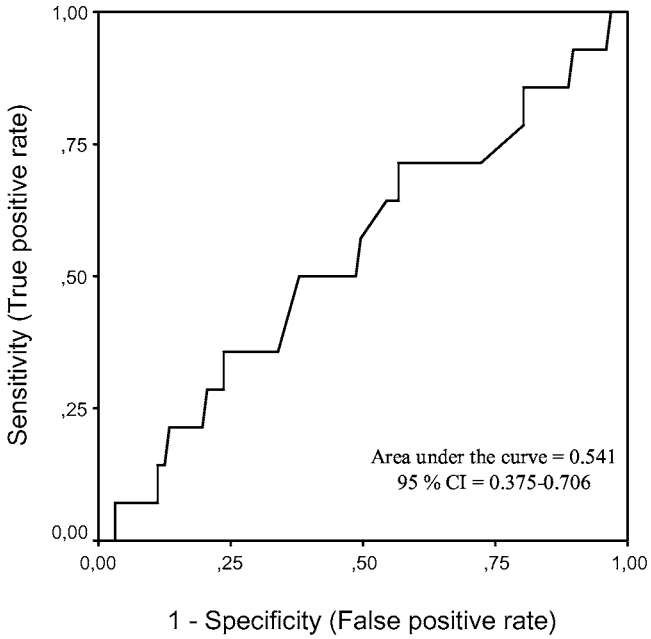

Figure 2. ROC curve showing sensitivity (true-positive rate) and 1-spesificity (false-positive rate) of individual chemotherapy score to predict mobilisation failure in each NHL patient.

5.3. Toxicity and efficacy of BEAC and BEAM regimens (Study III)

In BEAC group the number of $CD34^+$cells infused was 3.2 x 10^6/kg (1.0-12.0) and in BEAM group 3.1 x 10^6/kg (1.0-19.7) without statistical difference. In addition, no differences were observed in the time of engraftment according to the regimen used and or in the need for supportive care between these two patient groups. The peak values for CRP were higher in BEAM group and oral mucocitis was also more common. The data concerning infectious complications and regimen-related toxicity are also presented in Table 10. No differences in survival were observed between BEAC and BEAM groups. Kaplan-Meier estimates for OS (Figure 3) and PFS (Figure 4) for NHL patients conditioned with either BEAC or BEAM regimens are presented.

Table 10. Toxicity and supportive care data in NHL patients receiving BEAC or BEAM regimen.

	BEAC (N=36) Number (%)	BEAM (N=35) Number (%)	P value
Sepsis	3 (8)	10 (29)	0.062
Pneumonia	2 (6)	2 (6)	NS
Peak CRP mg/l (range)	113 (18-248)	140 (34-391)	0.034
Antibiotics, days (range)	9 (0-23)	9 (0-35)	NS
In-hospital days (range)	21 (18-64)	21,5 (16-43)	NS
Toxicity [#]			
Oral mucositis	10 (28)	21 (60)	0.015
Diarrhoea	3 (8)	10 (29)	0.062
Cardiac	2 (6)	0	NS
Renal	0	1 (3)	NS
Hepatic	0	2 (6)	NS
Pulmonary	1 (3)	0	NS
TRM	1 (3)	3 (9)	NS

Abbreviations: TRM, treatment-related mortality; CRP, C-reactive protein; NS, not significant; [#]only WHO grade > 2 (Miller et al. 1981) included

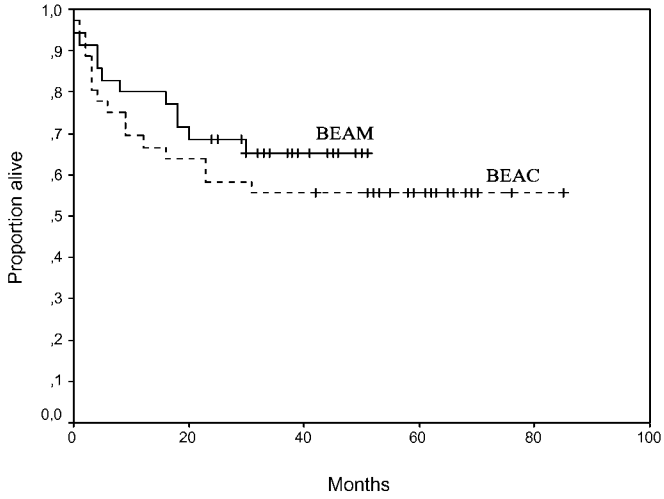

Figure 3. OS after HDT in NHL patients conditioned either with BEAC (N=36) or BEAM (N=35).

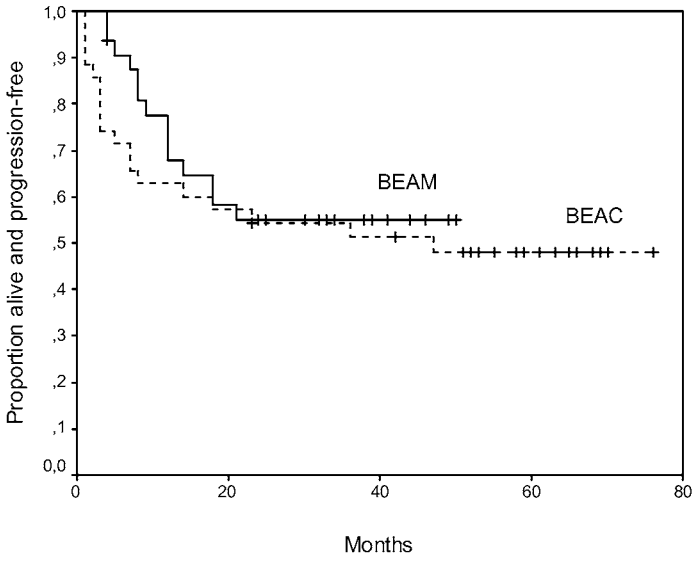

Figure 4. PFS after HDT in NHL patients conditioned either with BEAC (N=36) or BEAM (N=35).

5.4. Very acute cardiac toxicity during BEAC regimen (Study IV)

LA end-systolic area averaged 15.2 ± 1.2 cm^2 at baseline. It increased to 18.5 ± 1.4 cm^2 (P=0.001) during the study (Figure 5a). At the same time left ventricular end-diastolic volume (LVEDV) increased from 136.1 ± 12.3 cm^3 to 156.6 ± 11.1 cm^3 (P=0.04) (Figure 5b) and left ventricular end-systolic volume (LVESV) from 67.4 ± 7.8 cm^3 to 75.3 ± 7.1 cm^3 (P=0.018) (Figure 5c). However, there was no significant change in mean LVEF (from 51.0 ± 1.9 % to 51.3 ± 3.1 %, P=0.87). In addition, no change was observed in the LV mass (167.9 ± 11.7 g to 177.3 ± 9.2 g, P=0.1).

The baseline plasma concentration of NT-proANP was 481.1 ± 105.5 pmol/l. It increased during BEAC chemotherapy to 1056.6 ± 193.1 pmol/l (P=0.001) (Figure 6a). The plasma concentration of NT-proBNP was 134.9 ± 53.3 pmol/l at baseline and increased to 547.1 ± 168.4 pmol/l (P=0.003) (Figure 6b). At baseline there were seven patients (41 %) with LVEF < 50 % by MRI and during the study only one patient developed a significant

decrease in LVEF according to the definition used. Two patients died shortly after the study period because of sepsis and multiorgan failure (MOF).

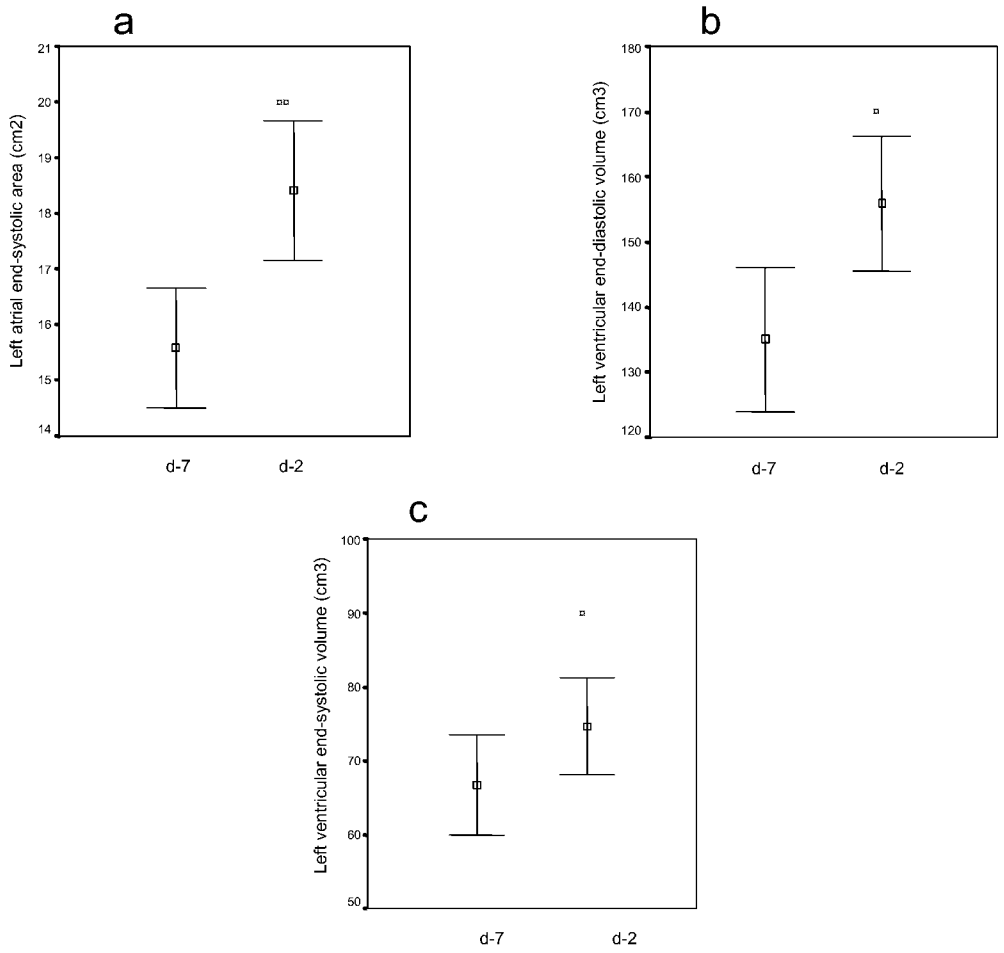

Figure 5. **(a)** Left atrial end-systolic area (cm^2), **(b)** Left ventricular end-diastolic volume (cm^3) **(c)** Left-ventricular end-systolic volume (cm^3) during BEAC regimen. Values are mean $\pm$ SEM (N=15). Significance of differences: ¤P<0.05, ¤¤P<0.01.

The change in NT-proBNP correlated significantly with the change in LVEDV (r=0.666, P=0.009) and a borderline correlation was found between the changes in NT-proBNP and LVESV (r=0.521, P=0.056). There was also a trend towards a correlation between the change in plasma concentration of NT-proBNP and in LVEF from d-7 to d-2 (r=-0.479, P=0.07).

Serum haemoglobin (Hb) level averaged 115 ± 9 g/l at baseline. It decreased to 98 ± 8 g/l (P<0.001) during the study. The serum creatinine level was 81 ± 10 µmol/l at baseline and decreased to 76 ± 10 µmol/l (P=0.009). In addition, no change was observed in HR (67 ± 17 to 68 ± 17, P=0.97). There was no significant change either in systolic blood pressure (119 ± 9 mmHg to 116 ± 10 mmHg, P=0.3) or diastolic blood pressure (71 ± 6 mmHg to 67 ± 8 mmHg, P=0.5). Neither SV (69 ± 21 cm^3 to 81 ± 33 cm^3, P=0.11) nor CO (4.3 ± 0.9 l to 4.9 ± 1.8 l, P=0.22) differed from baseline. Furthermore, no significant change in body weight (73 ± 11 kg to 74 ± 12 kg, P=0.15) was observed.

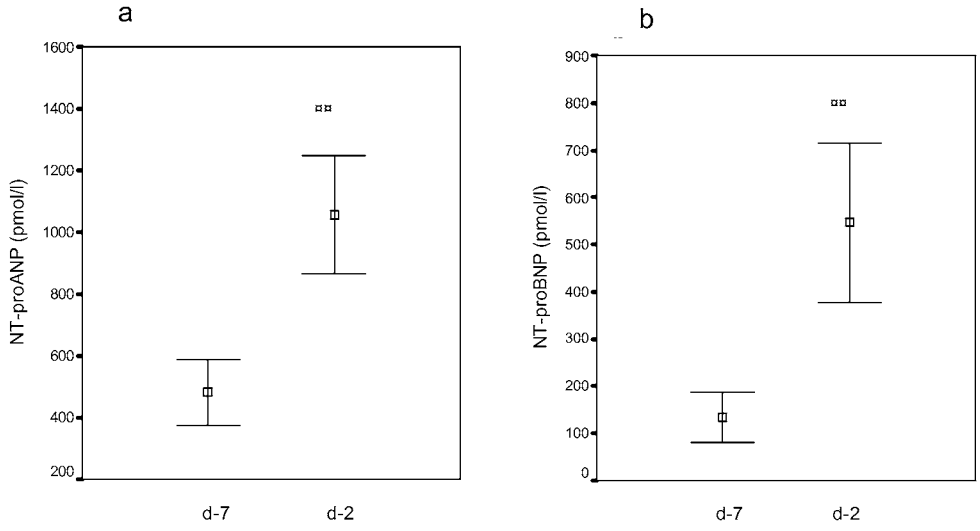

Figure 6. **(a)** Plasma NT-proANP values (pmol/l) and **(b)** Plasma NT-proBNP values (pmol/l) at baseline (d-7) and just after completing BEAC regimen (d-2). Values are ± SEM (N=17). Significance of differences: ¤P<0.05, ¤¤P<0.01.

5.5. Cardiac effects within three months of BEAC regimen (Study V)

Two patients developed severe sepsis and died because of MOF shortly after the stem cell infusion (d+7 and d+10, respectively). Thus, the early treatment-related mortality was 6 %. Only one patient (3 %) experienced clinical heart failure.

The mean LVEF was 53 ± 2 % at baseline. LVEF decreased significantly by d+12 (49 ± 2 %, P=0.009 vs. baseline). However, LVEF on m+3 (51 ± 2 %, P=0.134) did not differ from baseline (Figure 7a). At baseline nine patients (30 %) presented with systolic dysfunction. Moreover, systolic dysfunction was observed in 11 patients on d+12 (41 %) and also on m+3 (44 %).

The mean TTPF was 157 ± 8ms at baseline. TTPF no longer differed from baseline on d+12 (166 ± 7ms, P=0.75) and on m+3 (159 ± 6ms, P=0.85). Diastolic dysfunction was found in seven patients (23 %) at baseline. Altogether nine patients (33 %) presented with diastolic dysfunction on d+12 and two patients (8 %) on m+3. Four patients (13 %) had both systolic and diastolic dysfunction at baseline, nine (33 %) on d+12 and none on m+3.

The NT-proANP averaged 445 ± 65 pmol/l at baseline. NT-proANP increased significantly by d-2 (1127 ± 142 pmol/l, P<0.001) and thereafter started to decrease. NT-proANP was still significantly increased on d+7 (648 ± 125 pmol/l, P=0.015), but on d+12 (568 ± 111 pmol/l, P=0.081) and on m+3 (352 ± 28 pmol/l, P=0.556) the values no longer differed from baseline (Figure 7b). NT-proANP was elevated at baseline in eight (26 %), on d-2 in 28 (93 %), on d+7 in 11 (37 %), on d+12 in 12 (40 %) and on m+3 in four (17 %) patients.

The mean NT-proBNP was 129 ± 33 pmol/l at baseline. It increased by d-2 (624 ± 148 pmol/l, P<0.001) and thereafter began to decrease. On d+7 (404 ± 157 pmol/l, P=0.048). NT-proBNP was still significantly elevated, but on d+12 (268 ± 116 pmol/l, P=0.12) and on m+3 (68 ± 7 pmol/l, P=0.07) the values no longer differed from baseline (Figure 7c). At baseline NT-proBNP exceeded the upper normal limit in six (20 %), on d-2 in 24 (80 %), on d+7 in eight (27 %), on d+12 in five (19 %) and on m+3 in one (4 %) patients.

The mean body weight was 77 ± 2 kg at baseline. It increased significantly by d-2 (80 ± 3 kg, P=0.009). Thereafter it started to decrease (d+7, 78 ± 2 kg, P=0.051; d+12, 78 ± 2 kg, P=0.06) and on m+3 (76 ± 3 kg, P=0.056), body weight no longer differed from baseline. C-reactive protein (CRP) averaged 13 ± 5 mg/l at baseline. It increased significantly by d+7 (131 ± 14 mg/l, P<0.001). By d+12 it had decreased, but it still differed significantly from baseline (39 ± 5 mg/l, P=0.003). The mean haemoglobin (Hb) was 115 ± 2 g/l at baseline. It was significantly decreased on d-2 (101 ± 2 g/l, P<0.001), d+7 (92 ± 2 g/l, P<0.001) and d+12 (102 ± 2 g/l, P<0.001). Furthermore, serum creatinine level decreased from baseline (85 ± 3 µmol/l) to d-2 (79 ± 2 µmol/l, P=0.001). On d+7 (78 ± 2 µmol/l, P=0.003) and d+12 (80 ± 3 µmol/l, P=0.009), it still differed significantly from baseline. Systolic blood pressure showed a borderline significant decrease from d-7 to d+7 (125 ± 3 mmHg; 119 ± 2 mmHg, P=0.059). In addition, HR increased from baseline (66 ± 3) to d+7 (76 ± 3, P<0.001) and to d+12 (78 ± 4, P=0.001).

The possible effects of the extensive fluid infusion on natriuretic peptides levels were studied. The correlations in changes from d-7 to d-2 between body weight and ANP and BNP were significant (r=0.508, P=0.004; r=0.551, P=0.002, respectively). No other significant correlations were observed.

For LVEF, the group x time interaction was P=0.132, the group effect P<0.001 and time effect P=0.301. In addition, in TTPF the group x time interaction was P=0.110. Furthermore, neither the time effect P=0.313 nor the group effect P=0.996 were statistically significant. NT-proANP (Figure 8a) and NT-proBNP (Figure 8b) values in both subgroups are presented. When analysing natriuretic peptide values, the group x time interactions for NT-proANP and NT-proBNP were P=0.136 and P=0.445 and no group effects were seen (P=0.111; P=0.288, respectively). However, the time effects were significant (P<0.001; P<0.001, respectively). For the changes in NT-proANP, the group x time interaction was P=0.194, the time effect P<0.001 and the group effect P=0.074. Moreover, for the changes in NT-proBNP the group x time interaction was P=0.464, the time effect P<0.001 and the group effect P=0.328.

Because for all variables the group x time interaction was not significant, further analyses were not performed.

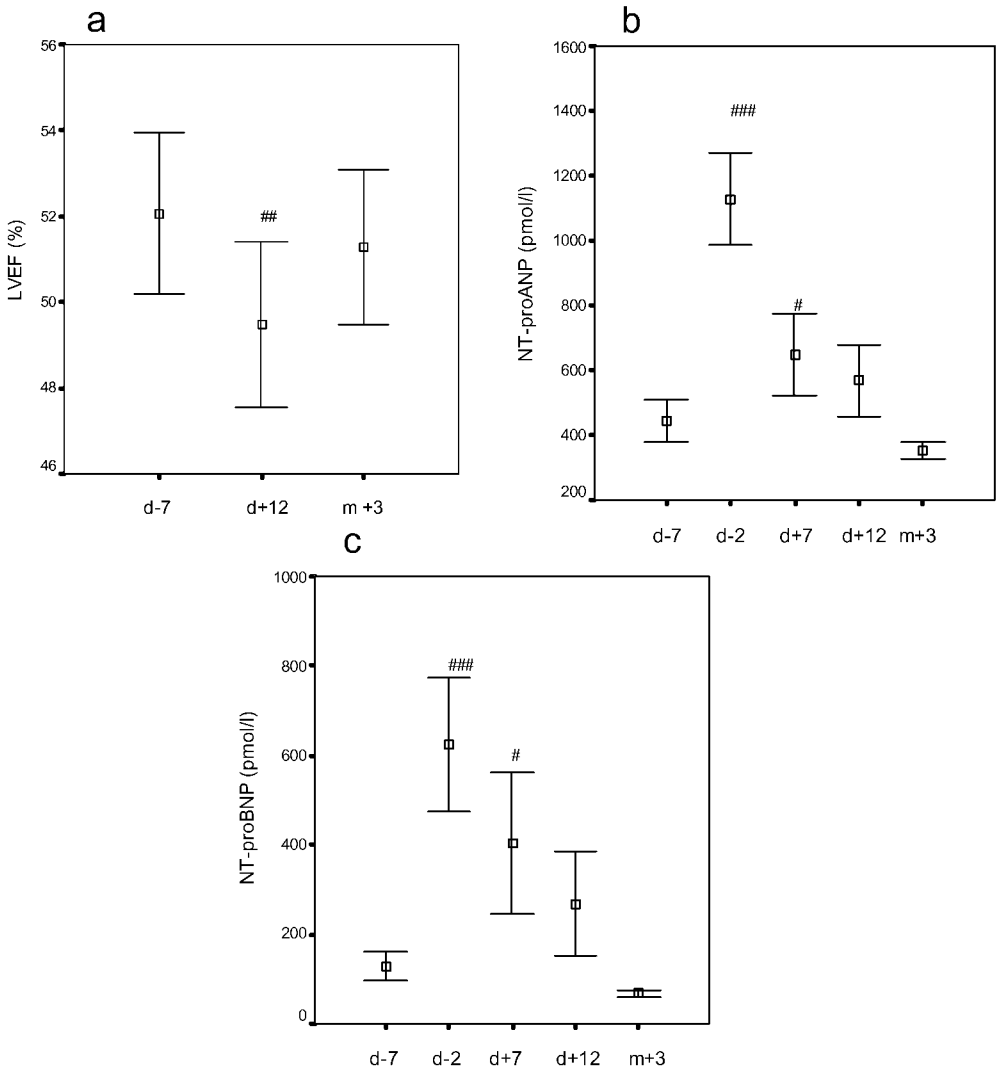

Figure 7. (a) Left ventricular ejection fraction (LVEF) **(b)** plasma NT-proANP and **(c)** plasma NT-proBNP at baseline d-7, on d-2, d+7, d+12 and m+3. Values are means ± SEM. Significance of differences: # P<0.05, ## P<0.01 ###<0.001 compared with baseline.

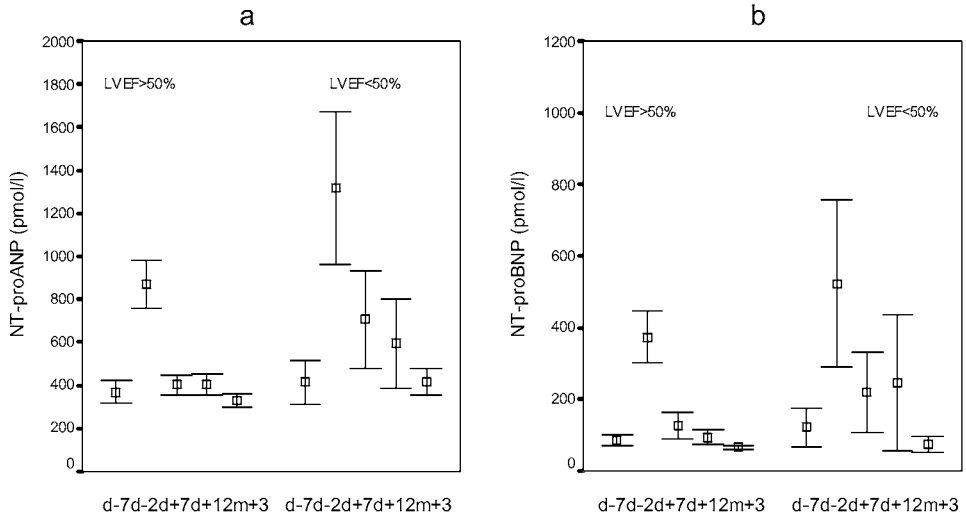

Figure 8. Patients with normal systolic function (LVEF $\geq$ 50 %) and with systolic dysfunction (LVEF < 50 %) at baseline. a) NT-proANP and b) NT-proBNP at baseline, and on d-2, d+7, d+12 and m+3. Values are means $\pm$ SEM.

5.6. Outcome of progressive disease after ASCT(StudyVI)

The median time from HDT to progression was seven months (1-66): four months (1-45) in DLBCL, seven months (1-38) in T-NHL, and eight months (1-59) in FL and 18 months (3-66) in MCL. The salvage therapies applied and their responses are presented in Table 11. Altogether 19 patients (17 %) receiving only supportive care (corticosteroids, blood transfusions) were excluded from the analyses. Most of them (73 %) had DLBCL. Altogether 12 patients (FCL=5, MCL=4, DLBCL=3) were treated with rituximab and two reveived also SCT (allogeneic=1, autologous=1). The objective response rate for rituximab containing salvage therapy was 83 % (CR 42 %).

Table 11. Characteristics and responses of salvage therapies in 96 NHL patients with progressive disease after ASCT.

	Number of patients (%)
Salvage therapy	
Chemotherapy only	50 (52)
Chemotherapy plus radiotherapy	17 (17)
Radiotherapy only	9 (9)
Rituximab with/or without chemotherapy	12 (13)
Chemotherapy followed by SCT	8 (8)
Allogeneic	5 (5)
2^{nd} ASCT	3 (3)
Response to salvage therapy	
CR	24 (25)
PR	30 (31)
NR	42 (44)
CR or PR to salvage therapy according to histology	
Diffuse large B-cell	15 (41)
Follicular	18 (69)
Mantle cell	10 (67)
T-cell	8 (72)
Other	3 (49)

Abbreviations: SCT, stem cell transplantation; ASCT, autologous stem cell transplantation; CR complete remission; PR partial remission; NR, non-responder

In univariate analysis several factors were associated with treatment response or survival but most of these factors lost their significance in multivariate analysis. Factors predicting treatment response after the progression included the use of rituximab (P=0.036), histology other than DLBCL (P=0.001) and IPI $\leq$ 2 at progression (P<0.001). Normal LDH at progression (P=0.002), response to salvage treatment (CR or PR) (P<0.001) and time from ASCT to progression $\geq$ 7 months (P=0.022) were predictors for overall survival.

The median OS for all patients was eight months and the estimated 4-year survival 21 % (Figure 9). The patients who progressed later than seven months after ASCT enjoyed a significantly better outcome compared to patients who progressed earlier, median 15 months (0-81) vs. two months (0-98) (P<0.001). The best OS was observed in patients with FL or MCL (Figure 10). Achievement of CR or PR with salvage therapy was associated

with better survival compared to non-responders (median 26.5 months vs. two months) (P<0.001). The OS was better for patients who received rituximab (median not reached); only patients receiving salvage therapy included when compared to those patients treated without rituximab (median ten months) (P=0.02). Furthermore, OS was significantly better in patients with both normal LDH at time of disease progression and time to progression $\geq$ 7 months from ASCT when compared to patients with both adverse factors (P<0.001) (Figure 11).

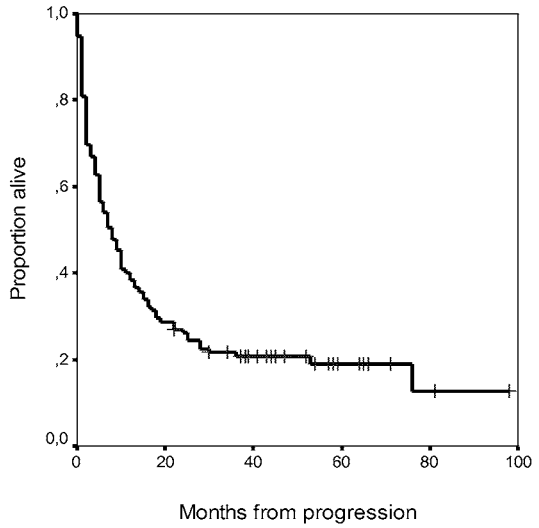

Figure 9. Overall survival of 115 NHL patients with progressive disease after ASCT.

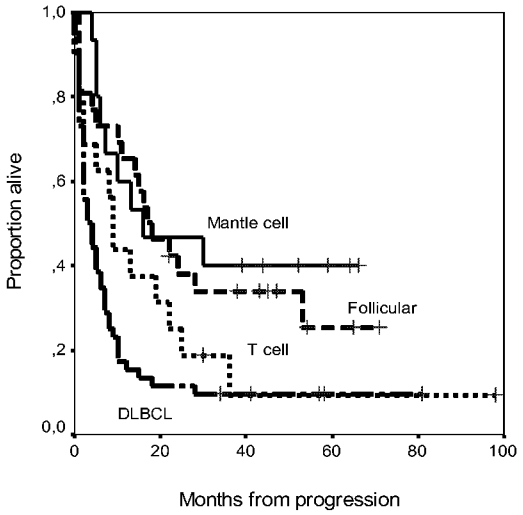

Figure 10. Overall survival of 115 NHL patients with progressive disease after ASCT by histology: diffuse large B cell (DLBCL) (N=52), follicular (N=26), T cell (N=16) and mantle cell (N=15).

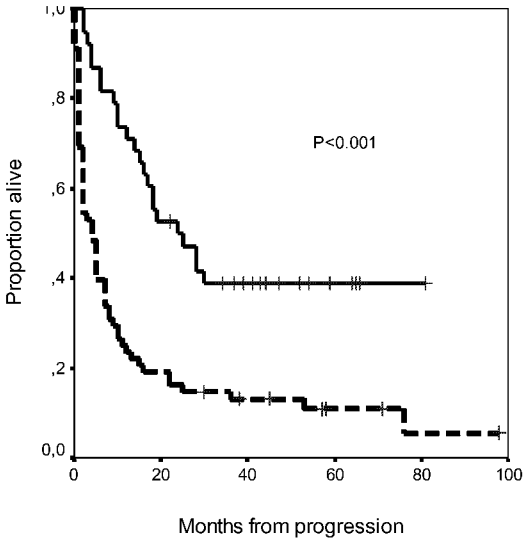

Figure 11. Overall survival in 115 NHL patients with progressive disease after ASCT according to two prognostic factors, LDH and time from HDT to progression. Upper curve: normal LDH and progression ≥ 7 months after ASCT (N=38). Lower curve: LDH > normal and progression < 7 months after ASCT (N=68). Log-rank P<0.001.

6. DISCUSSION

6.1. Patients and methods

The present series of studies is based on four retrospective studies (Studies I-III and VI) and a prospective trial (Studies IV-V). The participitants in all studies were adult (≥ 16 y) Finnish NHL patients, which makes the studies homogeneous in terms of malignant disease. The HPC mobilisation protocol was the same in all patients (Studies I-III) as was as the dose of CY in BEAC regimen (Studies IV-V). In study VI, the data was collected by a single observer (T.K.) limiting interindividual variation related to interpretation of clinical data.

The methods were based on widely accepted criteria for mobilisation success and the WHO toxicity scale (Miller et al. 1981). Also the definition for the NHL treatment response (CR/PR/NR) is commonly used. HPC collections were perfomed by the same aphaeresis machine, and the blood $CD34^+$ counts and aphaeresis $CD34^+$ cell yields were measured in the same stem cell laboratory (Studies I-III).

The prospective studies comprised only NHL patients treated with a single HDT regimen. Furthermore, the patients were monitored carefully. The study protocol was designed to assess cardiac function and neurohumoral activation simultaneously.

6.2. Prediction of haematopoietic progenitor cell mobilisation (Studies I-II)

Two premobilisation factors associated with mobilisation failure were found in this study: BM involvement at the time of diagnosis and platelet count just prior to the HPC mobilisation. Based on these two factors, a mathematical model for the prediction of mobilisation failure was constructed. Moreover, the original chemotherapy score (Drake et al. 1997) and the improved chemotherapy score could not predict mobilisation success in NHL patients. Therefore, the only obvious advantage of this improved scoring was that it was more widely applicable to those patients with NHL than the original one.

In Studies I-II, almost 20 % of NHL patients failed to mobilise an adequate numbers of progenitor cells to proceed to HDT which is in line with other studies (Ketterer et al. 1998,

Sugrue et al. 2000). The criterion for successful mobilisation was a collection of $\geq 1.5 \times 10^6$/kg $CD34^+$ cells. Many studies have established that the ideal PBPC $CD34^+$ cell dose for reliable engraftment is $\geq 5 \times 10^6$/kg with a target of $\geq 2.0 \times 10^6$/kg (Weaver et al. 1995, Glaspy et al. 1999). The minimum criterion for successful mobilisation used in this study seems to be valid, because all evaluable patients showed engraftment after HDT.

Several postmobilisation factors have been shown to predict mobilisation success (Sugrue et al. 2000, Kessinger et al. 2003). Also in this study, low platelet and WBC count nadirs and low peak B-$CD34^+$ counts were predictive of mobilisation failure in univariate analysis. The value of these factors is limited, because there is little that can be done at this point to avoid mobilisation failure. On the other hand, the prediction of mobilisation problems using premobilisation factors may allow starting HPC aphaeresis with lower B-$CD34^+$counts, because it is unlikely that very high counts will ensue.

BM involvement was an important factor affecting progenitor cell mobilisation in NHL patients and retained its predictive value also in multivariate analysis. BM involvement at diagnosis or prior to mobilisation has been found to be an important factor in NHL patients mobilised with G-CSF alone (Micallef et al. 2000). Apparently, marrow infiltration by malignant cells affects the microenvironment thereby making effective HPC mobilisation more difficult.

Low platelet count just prior to mobilisation was an important predictive factor for mobilisation failure. This finding has not been reported earlier in patients with NHL. Ketterer et al. (1998) studied patients with NHL, multiple myeloma and Hodgkin's disease and did not find the platelet count just prior to mobilisation to be a significant factor in predicting mobilisation success. Lower platelet counts may reflect poor marrow reserves or prolonged action of previous myelosuppressive therapy. Although no difference was observed in the time interval between the last chemotherapy and mobilisation between patients who were successful mobilisers and those who failed the mobilisation in this study, it is possible that patients with lower platelet counts had not yet fully recovered from the previous chemotherapy cycle. The importance of the time interval from chemotherapy to

mobilisation has been stressed especially in patients with FL (Perry et al. 1998) and in patients treated with more intensive chemotherapy (Tarella et al. 1999).

The mathematical model derived from the results of multivariate analysis showed a reasonable sensitivity. The area under the ROC curve (82 %) suggested that other yet unidentified factors might also be of importance in predicting HPC mobilisation. The pathophysiology of stem cell kinetics is only partly elucidated. Interindividual differences may be operating also in NHL patients mobilised with chemotherapy plus G-CSF, in analogy with healthy progenitor cell donors mobilised with G-CSF alone (Roberts et al. 1995). Vantelon et al. (2000) have proposed a mathematical model based on the number of moderately or highly myelotoxic chemotherapeutic regimens to predict the success of HPS mobilisation. Their model had relatively good predictive value with a success rate of 80 % in patients with low scores and below 20 % in patients with high scores.

Previous chemotherapy has an important influence on the success of progenitor cell mobilisation in patients with haematological malignancies (Haas et al 1994, Laszlo et al 2000, Vantelon et al. 2000). Most published series have been heterogeneous in regard to diagnoses, previous therapy and mobilisation protocol. Suprisingly the number of previous chemotherapy cycles was not predictive of mobilisation failure in this study (Study I). This suggests that in addition to quantity also the quality of previous chemotherapy might be an important issue influencing the stem cell pool.

Drake et al. (1997) have proposed a scoring system for previous chemotherapy to predict HPC mobilisation in patients with haematological malignancies. However, many commonly used drugs were missing from their scoring system. Because the original scoring system was applicable only in 27 % of the studied NHL patients, an improved chemotherapy scoring system was devised by adding several drugs to the original scoring. The improved scoring was applicable in 93 % of NHL patients (Study II). Only 2 % of patients had received TF 4 drugs, and none of them had received melphalan or carmustine, well-known stem cell toxins (Gardner et al. 1993b, Neben et al. 1993). This is the most apparent explanation for the lack of correlation between the chemotherapy score and the efficiency of HPC mobilisation. Perhaps the main reason for why the chemotherapy score did not predict

mobilisation is the fact that limited data are available on the toxicity of various drugs on progenitor cell pool. This is especially true when several drugs with variable modes of action are used concomitantly.

6.3. Toxicity and efficacy of BEAC and BEAM regimens (Study III)

BEAC and BEAM regimens possessed equal efficacy in NHL patients in terms of OS and PFS. On the other hand, BEAM was associated with higher incidence of oral and gastrointestinal toxicity. The differences in toxicity profiles of these regimens reflect the differences between side-effects of CY and melphalan.

No prospective randomised trials are available on the relative efficacy of various high-dose regimens in patients with NHL. A retrospective registry analysis suggested that TBI-based regimens are inferior to chemotherapy-only regimens in the treatment of histologically aggressive NHL (Salar et al. 2001). Also in that study no significant differences were observed in terms of OS between patients receiving BEAM or BEAC.

All patients were rescued by PBPC support after ASCT. Similar supportive care and routine administration of G-CSF were offered to all patients after HDT. These patient groups did not differ from each other in terms of various patient or disease-related characteristics or in the median number of PBPCs infused after HDT. This suggests that the finding of more prevalent oral and gastrointestinal toxicity associated with BEAM conditioning is a real finding and of possible clinical significance.

Severe mucositis and diarrhoea are major problems related to HDT (Rapoport et al. 1999). Mucosal damage during the aplastic period gives rise to bacteraemia with associated complications. The patients conditioned with BEAM had more prevalent mucositis with a grade > 2 in addition to more frequently positive blood cultures. In fact, all early treatment-related deaths in patients conditioned with BEAM were due to sepsis and MOF. Mucosal toxicity has been shown to be the main dose-limiting factor of high-dose melphalan (Samuels and Bitran 1995, Moreau et al. 1999). Furthermore, one prospective study yielded the highest scores for oral mucositis in patients receiving high-dose melphalan (Wardley et al. 2000). Caballero et al. (1997) reported grade 3-4 mucositis in 30 % of the patients after BEAM. In

fact, a somewhat higher prevalence for oral mucositis in patients who received BEAM was observed in this study. One explanation might be that the median age of the patients was higher in this study. Amifostine might protect the mucosa against melphalan-related toxicity without decreasing response rates (Capelli et al. 2000, Phillips et al. 2004). Recently a randomised study showed the efficacy of palifermin in terms of mucosal toxicity and its clinical consequenses in NHL patients reveiving TBI-based HDT (Spielberger et al. 2004). There might also be a need for such studies in patients receiving BEAM regimen.

Whereas BEAM seems to be somewhat more toxic regimen to the gastrointestinal tract, other non-haematological toxicities were rare in this analysis, with no apparent differences between BEAC and BEAM. Some investigators have reported significant toxicities associated with BEAC regimen (van Besien et al. 1995). Especially cardiac toxicity might be an important adverse event in patients receiving CY-containg regimens (Carlson et al. 1994, Jantunen et al. 2006).

6.4. Cardiac effects of BEAC regimen (Studies IV-V)

A novel finding was the significant enlargement of heart chambers immediately after completing BEAC regimen in NHL patients. This cardiac effect could not be assessed by MRI LVEF measurements, but it was clearly reflected by increased levels of natriuretic peptides (Study IV). A modest but significant impairment in systolic function was observed by RVG about two weeks after BEAC. However, the concomitantly measured natriuretic peptide values no longer differed from baseline. The changes both in cardiac systolic function and natriuretic peptide levels were transient returning towards baseline by three months. Thus, plasma natriuretic peptides seem to be more sensitive than LVEF to detect subclinical cardiac dysfunction (Study V).

A significant increase in natriuretic peptide levels just after completing BEAC was observed concomitantly with enlargement of the heart chambers. In addition, a strong correlation between the changes in NT-proBNP and LVEDV was found. This is logical, since natriuretic peptides are secreted as a result of dilatation of cardiac chambers. BNP is primarily released from the ventricles and reflects the filling pressure of left ventricle. Surprisingly, in Study IV, no significant changes were detectable either in LVEF or LV

mass. Furthermore, Hb as well as creatinine level decreased significantly while SV, CO and body weight showed no significant changes. Because intravenous diuretic boluses were administered during BEAC to keep the body weight stable, the stretch of atrial and ventricular walls by intensive fluid infusion can not explain all the changes in NT-proBNP.

In Study V during BEAC, there was a significant increase in both natriuretic peptide levels and body weight. In addition, the changes in natriuretic peptide values and body weight showed significant correlation. Furthermore, Hb levels decreased significantly that might be explained by increased venous capacitance and shifting of intravascular volume into the extravascular compartment due to increased permeability of the vascular endothelium and increased hydraulic pressure in the capillary bed. Perhaps the larger number of patients in Study V than in study IV (N=30 vs. N=17) partly explains these conflicting data.

This study demonstrated LV systolic dysfunction about two weeks (d+12) after BEAC regimen (Study V), which is in line with Gottdiener et al. (1981). In most previous studies the first cardiac function evaluation after baseline has been performed one month from HDT. The highest natriuretic peptide values were observed on d-2 but still on d+7 these values were elevated when compared to baseline. Thus, on d+12 natriuretic peptide levels were already returning to baseline. The increase in CRP and HR values is logical since between d+2 and d+7 all patients developed neutropenic fever and infections. The effect of neurohumoral activation due to sepsis might have some impact on these natriuretic peptide values. In addition, after completing BEAC the body weight remained stable due to intravenous boluses of diuretics. As discussed earlier, hyperhydration can not explain all these phenomena observed. A recent study showed that NT-proANP and NT-proBNP may serve as useful markers to indicate myocardial dysfunction and may help to differentiate between survivors and non-survivors of severe sepsis (Brueckmann et al. 2005). In study V, two patients died because of sepsis and MOF. They had 5-10-fold higher NT-proBNP values prior to septic shock than other patients at the same time point (data not shown).

Interestingly, we could not detect a significant change in LV diastolic function in this patient cohort. TTPF was used to assess cardiac diastolic function, which might be more

stable than another diastolic index, peak filling rate (Miller et al. 1986). There are two prospective studies in which the resting or exercise TTPF was prolonged even a year after HDT. However, patients with allogeneic stem cell transplantation were also included in these studies (Lele et al. 1996, Niwa et al. 2001). In Study IV, further data on LV wall thickness and stiffness and on LA volume would have revealed more information on cardiac diastolic function. Lim et al. (2006) showed that increased LA volume in patients with normal LV systolic function and suspected heart failure is a powerful independent predictor of LV diastolic dysfunction as predicted by serum NT-proBNP. In that study 2D echo was used to assess cardiac function. Furthermore, in study V, the atrial filling would have been specified by including the first-pass study to RVG method.

It has previously been suggested that changes in diastolic function precede changes in systolic function in response to anthracycline related cardiotoxicity (Cittadini et al. 1991) and that ANP might be an early marker of that cardiotoxicity (Daugaard et al. 2005). At baseline, systolic LV dysfunction was observed in nine (30 %) and diastolic dysfunction in six (20 %) patients (Study V). Subacute or late anthracycline cardiotoxicity might explain some of these baseline observations and perhaps also some of the dysfunction observed after BEAC. Even though a statistically significant difference in LVEF from baseline to three months after BEAC was not observed, there were eleven patients (44 %) with impaired systolic and two patients (8 %) with impaired diastolic function at that time point. Longer follow-up is needed to find out whether this observation has impact on the quality of life or cardiac complications in the long-term.

The precise mechanisms of high-dose CY associated cardiac toxicity are unknown. One possible explanation for the cardiotoxicity of high-dose CY is toxic endothelial damage, followed by extravasation of toxic metabolites. These toxic metabolities result in interstitial haemorrhage and oedema (Gottdiener et al. 1981), which may lead to decreased myocardial compliance and thus, diastolic dysfunction. In this study focusing on time during BEAC (d-7 to d-2) a significant enlargement of heart chambers was detected simultaneously by an increase in natriuretic peptide levels while LVEF remained unchanged. These findings suggest that high-dose CY leads to activation of compensatory mechanisms by which heart

tries to retain the systolic function (Study IV). After the acute phase, cardiac reserves might be exhausted and dysfunction becomes evident (Study V). The measurement of neurohumoral markers like norepinephrine, which is considered as an early indicator of sympathetic activity, might better reveal the development of congestive heart failure (Nousiainen et al. 2001).

Neuroendocrine activation during or after HDT regimens has been studied earlier. Snowden et al. (2000) have reported that plasma BNP may be used for early detection of cardiac dysfunction during SCT. In their study, however, the peak levels of BNP occurred 1-4 weeks after HDT, which is not a very acute finding compared with the significant increase in natriuretic peptides already during the high-dose CY observed in this study. Niwa et al. (2001) showed an elevation on natriuretic peptides very shortly after HDT on day +1 and +14, but neither peak was predictive of later cardiac toxicity. In these pioneer studies the cardiac function tests were performed at baseline and in the weeks after HDT but not concomitantly with natriuretic peptide measurements. In the most recent study, the persistent and very acute increase in NT-proBNP after HDT was strongly associated with development of subclinical cardiac dysfunction as assessed by 2D echo (Sandri et al. 2005). However, cardiac function evaluation was performed 4 and 12 months after HDT and various HDT regimens were used. In addition, all these studies included patients with allogeneic stem cell transplants. Acute graft-versus-host disease and possible late infections may, thus partly, explain the neurohumoral activation observed.

Hertenstein et al. (1994) have suggested that patients with impaired LVEF at baseline should not be excluded from HDT protocols solely due to this finding. Most patients with baseline LVEF < 50 % did well in this study, which is in line with some previous studies (Bearman et al. 1990, Zangari et al. 1999, Brockstein et al. 2000). In addition, there were four patients with LVEF < 40 % at baseline, of whom two died because of sepsis and MOF (Study V). Patients with initially decreased LVEF may benefit from afterload reduction starting prior to HDT. ACE inhibitors have already been used in prophylactic treatment in patients with reduced LVEF prior to HDT (Kakavas et al. 1995).

Besides high-dose CY, BEAC regimen includes etoposide, cytarabine and carmustine. The risk of high-dose CY associated cardiac toxicity might be increased by concomitant administration of mitoxantrone or high-dose cytarabine (i.e 1-3 g/m^2) (Vaickus and Letendre, 1984). In this study, however, mitoxantrone was not used and the dose of cytarabine was lower. The minor impact of DMSO on the cardiac effects observed can not be ruled out.

6.5. Outcome of progressive disease after ASCT (Study VI)

Histology other than DLBCL, IPI ≤ 2 at time of progression and the use of rituximab in salvage therapy predicted treatment response for progressive disease after ASCT. In addition, normal LDH at the time of disease progression and longer time to progression after ASCT (≥ 7 months) were the most powerful predictors for survival.

Altogether 83 % of the patients who progressed after ASCT received salvage therapy. The response rate was relatively high (56 %), but was dependent on histology (DLBCL vs. others), use of rituximab and most significantly IPI at the time of disease progression. Although time from ASCT to progression was also of importance in univariate analysis, it lost its significance in multivariate analysis, apparently because patients with DLBCL tended to progress early. The prognosis of these patients is in general poor with current therapies. Moreover, many of these patients poorly tolerate current salvage treatments, as shown also in other series (Johnsen et al. 1996, Buchler et al. 2003).

Only a minority of the patients (N=12) reveived monoclonal antibody, because it became applicable in clinical practise in 1998. The use of rituximab as salvage therapy was associated with an improved response rate and improved survival in this study. However, there were nine patients out of twelve either with MCL or FCL subtype.The improved outcome is in line with observations by Kewalramani et al. (2003), whose report included only patients with aggressive NHL histology. In the treatment of newly diagnosed DLBCL rituximab has proved its efficacy (Coiffier et al. 2002). Also in the first-line treatment of FL and MCL, there are several reports showing the benefit of rituximab (Geisler et al. 2004, Hiddeman et al. 2005, Marcus et al. 2005). Furthermore, the use of rituximab should be

considered as a part of salvage therapy in patients with $CD20^+$ NHL who progress after ASCT (Pan et al. 2002). Because the number of rituximab-treated patients was very low in this study (13 %), a selection bias cannot be ruled out.

The median survival after progression was only eight months in this series, which is in agreement with other reports from single centres (Vose et al. 1992, Kewalramani et al. 2003, Paltiel et al. 2003). Moreover, the median survival was only four months in patients with DLBCL, of whom only 10 % were long-term survivors. On the other hand, the prognosis was much better in patients with MCL or those with FL. These patients could be effectively treated in most cases, but innovative treatments are also needed in these patients to improve the response duration. These may include, in addition to rituximab, RIC with allografting to promote GVL activity. A second ASCT may be considered in selected patients who progress several years after ASCT, although this approach is associated with marked early TRM (De Lima et al. 1997, Lenain et al. 2004). FL and MCL patients might be the best candidates for RIC allogeneic SCT, because the GVL effect is the most prominent in indolent lymphomas (Khouri et al. 1999).

Predictive factors for OS included the time from ASCT to progression, LDH at the time of progression and, most importantly, the response to salvage therapy. This is perhaps due to the fact that almost half of the patients had DLBCL, which is associated with very poor survival if remission is not obtained. Longer time to progression and normal LDH reflect less aggressive tumour biology and are also of prognostic value.

NHL patients who progress after ASCT are very heterogeneous in regard to their general condition, co-morbidities and ability to tolerate various treatments. These patient-related factors are of crucial importance for the evaluation of treatment options in a given patient. Disease-related factors like histology, IPI and time from ASCT to progression are also very important, as shown in this analysis. There are patients with aggressive histology, who progress early with high IPI and perhaps do not benefit from current standard therapeutic approaches. These patients may be treated with palliation only or, preferably, in prospective studies evaluating novel treatments. On the other hand, patients with DLBCL

who progress years after ASCT and those with FL or MCL can be salvaged successfully in many cases.

6.6. Study limitations

Four studies were retrospective (I-III, VI) even though studies I-III were based on prospectively collected data. Two different BEAM regimens were applied because of clinical practise (Study III). Moreover, the assessment of oral mucositis and diarrhoea was based on daily nursing reports and clinical examination by a treating physician. Because only > grade 2 toxicities were taken into account, the method used reflects only roughly severe toxicities related to HDT regimens (Study III).

In study IV, the patient population was small (N=17), which might have affected the results. The major finding, i.e. the substantial increase in natriuretic peptide levels, was observed in all patients and the changes were statistically significant. However, a novel method, MRI, was used. The study protocol was also quite demanding for the participating patients. Quantification of the LV volumes with a 3D data set instead of a geometric model, the modified Simpson's rule, might have given more accurate data because of three-dimensional approach for non-symmetric ventricles. However, the reproductability of the modified Simpson's method was established earlier in the study centre in favour of this method. Systolic dysfunction by MRI was defined as LVEF < 50 % which is lower than generally used (Salton et al. 2002) and was derived from RVG method.

In studies IV and V, all patients had previously received anthracyclines and there were five patients (17 %) with known cardiovascular disease (Study V). It is difficult, however, to study NHL patients undergoing ASCT without prior exposure to anthracyclines, because the gold standard in first-line treatment for the most common ASCT indication, DLBCL, is anthracycline based therapy. During BEAC regimen hyperhydration was used. Moreover, all patients (Study V) developed neutropenic fever after d+2 and the engraftment was supported by G-CSF. These factors might have influenced the results. Furthermore, the cardiac function method (RVG) was not performed concomitantly with natriuretic peptide measurements either on d-2 or on d+7.

6.7. Concluding remarks

In this study different aspects of HDT supported by ASCT in adult NHL patients were evaluated. HPC mobilisation is a crucial part of ASCT, and factors affecting HPC mobilisation should be taken into account in the early treatment phase. A simple solution to poor mobilisation is unlikely to exist, because the reasons are multifactorial and obviously differ from one patient to another. Those NHL patients who have BM infiltration at diagnosis and lower platelet counts just prior to mobilisation are at a significant risk of mobilisation failure. Poor mobilisers are likely to benefit from novel mobilisation approaches, including AMD3100 (Broxmeyer et al. 2005) and SDF-1 analogues (Zhong et al. 2004). A recent study reported that sympathetic nervous system regulates the egress of stem and progenitor cells from their niche (Katayama et al. 2006). These results suggest that modulation of the sympathetic outflow to the stem cell niche might represent a novel HPC mobilisation method.

Any chemotherapy scoring system seems to be arbitrary and at best only partly reflects reality. Until more information is available on safety of chemotherapy for HPCs, it is advisable to avoid prolonged treatment with TF 3 and TF4 drugs in NHL patients who will undergo ASCT. It might also be useful to limit the use of less toxic drugs before HPC mobilisation. In the future new chemotherapeutic agents may be more targeted and also less toxic to HPCs, thus decreasing the risk of mobilisation failure.

BEAC and BEAM regimens showed comparable antitumour activity in patients with NHL, but BEAM was associated with increased incidence of mucosal damage. However, prospective randomised trials are definitively needed to draw firm conclusions on the relative toxicity and efficacy of various HDT regimens in NHL patients. Reduction of the mucosal toxicity of conditioning regimens seems to be a major issue in the future. The use of keratinocyte growth factor (palifermin) to prevent mucosal damage after HDT seems to be a promising approach, especially in high-risk patients.

High-dose CY seems to result in subclinical, transient systolic dysfunction. LVEF might not be sensitive enough for its detection. On the other hand, MRI is a useful method for showing very early cardiac toxicity, cardiac dilatation. A clinically relevant strategy to

assess and follow-up cardiac LV function seems to be serial measurements of natriutretic peptides. Patients with systolic dysfunction prior to high-dose CY may benefit from closer monitoring during BEAC and perhaps heart failure therapy. An issue for future trial could be the comparison of prophylactic heart failure therapy (ACE inhibitor, carvedilol or angiotensin II-receptor blocker) in a prospective fashion.

In the future, tissue or colour tissue Doppler 2D echo might be a useful tool in the assessment of LV function and structure in cancer patients undergoing ASCT. Strain means tissue deformation in response to an applied force. Preliminary studies suggest that strain rate may be superior to myocardial velocities especially in relaxation abnormalities. The application of strain rate awaits further refinements and wider availability (Quinones 2005). Comparison of cardiac function related to HDT by 3D MRI, tissue Doppler 2D and even 3D echo would be interesting.

For the future, studies in larger cohorts of patients and studies that compare newer functional and biochemical markers (e.g. endothelin-1 and proinflammatory cytokines) of early cardiac toxicity with conventional methods are needeed. These studies should help to establish the best tool to accurately identify patients at risk of developing clinical heart failure and to identify cardiac damage at the preclinical stage. This would allow clinicians to more closely monitor cardiovascular function, prevent cardiac function impairment and start treatment at an early phase in high-risk patients.

The outcome of NHL patients who progress after ASCT is generally poor, as was also shown in this study. However, there is a group of patients whose survival seems to be promising. Simple prognostic factors found in this study are helpful in determining the likelihood for the treatment response. These factors are also apparently useful when selecting NHL patients for prospective trials testing new or improved treatments. Such studies appear to be of paramount importance especially, in patients with DLBCL.

7. SUMMARY

Study I: BM involvement at diagnosis and suboptimal platelet count just prior to mobilisation are factors predicting mobilisation failure in NHL patients mobilised with intermediate-dose CY plus G-CSF.

Study II: Neither the original chemotherapy scoring nor the improved scoring system predicts HPC mobilisation in NHL patients mobilised with intermediate-dose CY plus G-CSF.

Study III: No significant difference was observed in the efficacy of BEAC and BEAM regimens. In NHL patients BEAM regimen seems to cause more oral mucositis and gastrointestinal toxicity with associated infections.

Stydy IV: BEAC regimen results in very acute cardiac toxicity characterised by cardiac dilatation in NHL patients as assessed with MRI. This cardiac effect can be observed with elevated natriutretic peptide values but not with measurement of LVEF.

Study V: BEAC regimen seems to cause acute transient LV systolic dysfunction observed by RVG. Natriuretic peptides might be more sensitive than LVEF to reflect this cardiac effect. Serial measurement of natriuretic peptides could offer a useful tool in the assessment and follow-up of cardiac systolic dysfunction in ASCT recipients.

Study VI: Histology other than DLBCL and IPI $\leq$ 2 are factors predicting treatment response for progressive disease after ASCT in NHL patients. Normal LDH at progression, longer time from ASCT to progression and response to salvage therapy are the most powerful predictors for overall survival.

8. REFERENCES

A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 1993;329:987-994.

Adams JE 3rd, Bodor GS, Davila-Roman VG, et al. Cardiac troponin I. A marker with high specificity for cardiac injury. Circulation 1993;88:101-106.

Adams MJ, Lipshultz SE, Schwartz C, Fajardo LF, Coen V, Constine LS. Radiation-associated cardiovascular disease: manifestations and management. Semin Radiat Oncol 2003;13:346-356.

Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC. The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. J Exp Med 1997;185:111-120.

Akahori M, Nakamae H, Hino M, et al. Electrocardiogram is very useful for predicting acute heart failure following myeloablative chemotherapy with hematopoietic stem cell transplantation rescue. Bone Marrow Transplant 2003;31:585-590.

Ala-Kopsala M, Magga J, Peuhkurinen K, et al. Molecular heterogeneity has a major impact on the measurement of circulating N-terminal fragments of A- and B-type natriuretic peptides. Clin Chem 2004;50:1576-1588.

Alizadeh A, Eisen M, Davis R, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503-511.

Andersen N, Pedersen L, Elonen E, et al. Primary treatment with autologous stem cell transplantation in mantle cell lymphoma: outcome related to remission pretransplant. Eur J Haematol 2003;71:73-80.

Ando M, Yokozawa T, Sawada J, et al. Cardiac conduction abnormalities in patients with breast cancer undergoing high-dose chemotherapy and stem cell transplantation. Bone Marrow Transplant 2000;25:185-189.

Andreadis C, Schuster SJ, Chong EA, et al. Long-term event-free survivors after high-dose therapy and autologous stem-cell transplantation for low-grade follicular lymphoma. Bone Marrow Transplant 2005;36:955-961.

Apostolidis J, Gupta R, Grenzelias D, et al. High-dose therapy with autologous bone marrow support as consolidation of remission in follicular lymphoma: long-term clinical and molecular follow-up. J Clin Oncol 2000;18:527-536.

Appelbaum F, Strauchen JA, Graw RG, et al. Acute lethal carditis caused by high-dose combination chemotherapy. A unique clinical and pathological entity. Lancet 1976;1:58-62.

Appelbaum FR. The use of bone marrow and peripheral blood stem cell transplantation in the treatment of cancer. CA Cancer J Clin 1996;46:142-164.

Armitage JO. Emerging applications of recombinant human granulocyte-macrophage colony-stimulating factor. Blood 1998;92:4491-4508.

Armitage S, Hargreaves R, Samson D, Brennan M, Kanfer E, Navarrete C. CD34 counts to predict the adequate collection of peripheral blood progenitor cells. Bone Marrow Transplant 1997;20:587-591.

Auner HW, Tinchon C, Brezinschek RI, et al. Monitoring of cardiac function by serum cardiac troponin T levels, ventricular repolarisation indices, and echocardiography after conditioning with fractionated total body irradiation and high-dose cyclophosphamide. Eur J Haematol 2002;69:1-6.

Aurlien E, Holte H, Kvaloy S, Jakobsen E, Rusten LS, Kvalheim G. Combination chemotherapy containing mitoguazone, ifosfamide, methotrexate, etoposide (MIME) and G-CSF efficiently mobilize peripheral blood progenitor cells in heavily pre-treated relapsed lymphoma patients. Eur J Haematol Suppl 2001;64:14-20.

Ayash LJ, Wright JE, Tretyakov O, et al. Cyclophosphamide pharmacokinetics: correlation with cardiac toxicity and tumor response. J Clin Oncol 1992;10:995-1000.

Bea S, Zettl A, Wright G, et al. Diffuse large B-cell lymphoma subgroups have distinct genetic profiles that influence tumor biology and improve gene-expression-based survival prediction. Blood 2005;106:3183-3190.

Bearman SI, Petersen FB, Schor RA, et al. Radionuclide ejection fractions in the evaluation of patients being considered for bone marrow transplantation: risk for cardiac toxicity. Bone Marrow Transplant 1990;5:173-7.

Bellenger NG, Burgess MI, Ray SG, et al. Comparison of left ventricular ejection fraction and volumes in heart failure by echocardiography, radionuclide ventriculography and cardiovascular magnetic resonance; are they interchangeable? Eur Heart J 2000; 21:1387-1396.

Bensinger W, Appelbaum F, Rowley S, et al. Factors that influence collection and engraftment of autologous peripheral-blood stem cells. J Clin Oncol 1995;13:2547-2555.

Benson AB 3rd, Ajani JA, Catalano RB, et al. Recommended guidelines for the treatment of cancer treatment-induced diarrhea. J Clin Oncol 2004;22:2918-26.

Benvenuto GM, Ometto R, Fontanelli A, et al. Chemotherapy-related cardiotoxicity: new diagnostic and preventive strategies. Ital Heart J 2003;4:655-667.

Bettencourt P. Clinical usefulness of B-type natriuretic peptide measurement: present and future perspectives. Heart Fail 2005;91:1489-1494.

Bigger JT Jr, La Rovere MT, Steinman RC, et al. Comparison of baroreflex sensitivity and heart period variability after myocardial infarction. J Am Coll Cardiol 1989;14:1511-1518.

Blystad AK, Enblad G, Kvaloy S, et al. High-dose therapy with autologous stem cell transplantation in patients with peripheral T cell lymphomas. Bone Marrow Transplant 2001;27:711-716.

Bonow R, Bacharach S, Green M, et al. Impaired left ventricular diastolic filling in patients with coronary artery disease: assessment with radionuclide angiography. Circulation 1981;64:315-323.

Boomsma F, van den Meiracker AH. Plasma A- and B-type natriuretic peptides: physiology, methodology and clinical use. Cardiovasc Res 2001;51:442-449.

Branson K, Chopra R, Kottaridis PD, et al. Role of nonmyeloablative allogeneic stem-cell transplantation after failure of autologous transplantation in patients with lymphoproliferative malignancies. J Clin Oncol 2002;20:4022-4031.

Braverman AC, Antin JH, Plappert MT, Cook EF, Lee RT. Cyclophosphamide cardiotoxicity in bone marrow transplantation: a prospective evaluation of new dosing regimens. J Clin Oncol 1991;9:1215-1223.

Brockstein BE, Smiley C, Al-Sadir J, Williams SF. Cardiac and pulmonary toxicity in patients undergoing high-dose chemotherapy for lymphoma and breast cancer: prognostic factors. Bone Marrow Transplant 2000;25:885-94.

Broxmeyer HE, Orschell CM, Clapp DW, et al. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. J Exp Med 2005;201:1307-1318.

Brueckmann M, Huhle G, Lang S, et al. Prognostic value of plasma N-terminal pro-brain natriuretic peptide in patients with severe sepsis. Circulation 2005;112:527-534.

Brunken RC, Schelbert HR. Positron emission tomography in clinical cardiology. Cardiol Clin 1989;7:607-629.

Buchler T, Hermosilla M, Ferra C, et al. Outcome and toxicity of salvage treatment on patients relapsing after autologous hematopoietic stem cell transplantation-experience from a single center. Hematology 2003;8:145-50.

Buja G, Miorelli M, Turrini P, Melacini P, Nava A. Comparison of QT dispersion in hypertrophic cardiomyopathy between patients with and without ventricular arrhythmias and sudden death. Am J Cardiol 1993;72:973-976.

Caballero MD, Rubio V, Rifon J, et al. BEAM chemotherapy followed by autologous stem cell support in lymphoma patients: analysis of efficacy, toxicity and prognostic factors. Bone Marrow Transplant 1997;20:451-458.

Capelli D, Santini G, De Souza C, et al. Amifostine can reduce mucosal damage after high-dose melphalan conditioning for peripheral blood progenitor cell autotransplant: a retrospective study. Br J Haematol 2000;110:300-307.

Cardinale D, Sandri MT, Martinoni A, et al. Left ventricular dysfunction predicted by early troponin I release after high-dose chemotherapy. J Am Coll Cardiol 2000;36:517-522.

Cardinale D, Sandri MT, Martinoni A, et al. Myocardial injury revealed by plasma troponin I in breast cancer treated with high-dose chemotherapy. Ann Oncol 2002;13:710-715.

Cardinale D, Sandri MT, Colombo A, et al. Prognostic value of troponin I in cardiac risk stratification of cancer patients undergoing high-dose chemotherapy. Circulation 2004;109:2749-2754.

Carlo-Stella C, Di NM, Milani R, Guidetti A, et al. Use of recombinant human growth hormone (rhGH) plus recombinant human granulocyte colony-stimulating factor (rhG-CSF) for the mobilization and collection of CD34+ cells in poor mobilizers. Blood 2004;103:3287-3295.

Carlson K, Smedmyr B, Backlund L, Simonsson B. Subclinical disturbances in cardiac function at rest and in gas exchange during exercise are common findings after autologous bone marrow transplantation. Bone Marrow Transplant 1994;14:949-954.

Carreras E, Bertz H, Arcese W, et al. Incidence and outcome of hepatic veno-occlusive disease after blood or marrow transplantation: a prospective cohort study of the European Group for Blood and Marrow Transplantation. Blood 1998;92:3599-3604.

Cazin B, Gorin NC, Laporte JP, et al. Cardiac complications after bone marrow transplantation. A report on a series of 63 consecutive transplantations. Cancer 1986; 57:2061-2069.

Chen H, Burnett J. Natriuretic peptides in the pathophysiology of congestive heart failure. Curr Cardiol Rep 2000;2:198-205.

Cheson BD. The role of radioimmunotherapy with yttrium-90 ibritumomab tiuxetan in the treatment of non-Hodgkin lymphoma. BioDrugs 2005;19:309-322.

Cittadini A, Fazio S, D'Ascia C, et al. Subclinical cardiotoxicity by doxorubicin: a pulsed Doppler echocardiographic study. Eur Heart J 1991;12:1000-1005.

Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002;346:235-242.

Colvin M, Hilton J. Pharmacology of cyclophosphamide and metabolites. Cancer Treat Rep 1981;3:89-95.

Constantine G, Shan K, Flamm SD, Sivananthan MU. Role of MRI in clinical cardiology. Lancet 2004;363:2162-2171.

Craig JI, Anthony RS, Stewart A, Thomson EB, Gillon J, Parker AC. Peripheral blood stem cell mobilization using high-dose cyclophosphamide and G-CSF in pretreated patients with lymphoma. Br J Haematol 1993;85:210-212.

Cullen M, Steven N, Billingham L, et al. Simple Investigation in Neutropenic Individuals of the Frequency of Infection after Chemotherapy +/- Antibiotic in a Number of Tumours (SIGNIFICANT) Trial Group. Antibacterial prophylaxis after chemotherapy for solid tumors and lymphomas. N Engl J Med 2005;353:988-998.

Daugaard G, Lassen U, Bie P, et al. Natriuretic peptides in the monitoring of anthracycline induced reduction in left ventricular ejection fraction. Eur J Heart Fail 2005;7:87-93.

Davis TA, Grillo-Lopez AJ, White CA, et al. Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin's lymphoma: safety and efficacy of re-treatment. J Clin Oncol 2000;18:3135-3143.

Dawson MA, Schwarer AP, Muirhead JL, et al. Successful mobilization of peripheral blood stem cells using recombinant human stem cell factor in heavily pretreated patients who have failed a previous attempt with a granulocyte colony-stimulating factor-based regimen. Bone Marrow Transplant 2005; 36:389-396.

Day CP, McComb JM, Campbell RW. QT dispersion: an indication of arrhythmia risk in patients with long QT intervals. Br Heart J 1990;63:342-344.

Deconinck E, Foussard C, Milpied N, et al. High-dose therapy followed by autologous purged stem-cell transplantation and doxorubicin-based chemotherapy in patients with advanced follicular lymphoma: a randomized multicenter study by GOELAMS. Blood 2005;105:3817-3823.

DeLeve LD. Cellular target of cyclophosphamide toxicity in the murine liver: role of glutathione and site of metabolic activation. Hepatology 1996;24:830-837.

de Lima M, van Besien KW, Giralt SA, et al. Bone marrow transplantation after failure of autologous transplant for non-Hodgkin's lymphoma. Bone Marrow Transplant 1997;19:121-127.

Demirer T, Buckner CD, Bensinger WI. Optimization of peripheral blood stem cell mobilization. Stem Cells 1996;14:106-116.

Demirer T, Ayli M, Ozcan M, et al. Mobilization of peripheral blood stem cells with chemotherapy and recombinant human granulocyte colony-stimulating factor (rhG-CSF): a randomized evaluation of different doses of rhG-CSF. Br J Haematol 2002;116:468-474.

Devine SM, Flomenberg N, Vesole DH, et al. Rapid mobilization of CD34+ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. J Clin Oncol 2004;22:1095-1102.

Drake M, Ranaghan L, Morris TC, et al. Analysis of the effect of prior therapy on progenitor cell yield: use of a chemotherapy scoring system. Br J Haematol 1997;98:745-749.

Dreyling M, Lenz G, Hoster E, et al. Early consolidation by myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission significantly prolongs progression-free survival in mantle-cell lymphoma: results of a prospective randomized trial of the European MCL Network. Blood 2005;105:2677-2684.

Dulce MC, Mostbeck GH, Friese KK, Caputo GR, Higgins CB. Quantification of the left ventricular volumes and function with cine MR imaging: comparison of geometric models with three-dimensional data. Radiology 1993;188:371-376.

Escalon MP, Champlin RE, Saliba RM, et al. Nonmyeloablative allogeneic hematopoietic transplantation: a promising salvage therapy for patients with non-Hodgkin's lymphoma whose disease has failed a prior autologous transplantation. J Clin Oncol 2004;22:2419-2423.

Farrell CL, Rex KL, Chen JN, et al. The effects of keratinocyte growth factor in preclinical models of mucositis. Cell Prolif 2002; 35: 78-85.

Feinstein LC, Sandmaier BM, Maloney DG, et al. Allografting after nonmyeloablative conditioning as a treatment after a failed conventional hematopoietic cell transplant. Biol Blood Marrow Transplant 2003;9:266-272.

Fitoussi O, Perreau V, Boiron JM, et al. A comparison of toxicity following two different doses of cyclophosphamide for mobilization of peripheral blood progenitor cells in 116 multiple myeloma patients. Bone Marrow Transplant 2001;27:837-842.

Flomenberg N, Devine SM, DiPersio JF, et al. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. Blood 2005;106:1867-1874.

Foran JM, Apostolidis J, Papamichael D, et al. High-dose therapy with autologous haematopoietic support in patients with transformed follicular lymphoma: a study of 27 patients from a single centre. Ann Oncol 1998;9:865-869.

Forrest DL, Nevill TJ, Naiman SC, et al. Second malignancy following high-dose therapy and autologous stem cell transplantation: incidence and risk factor analysis. Bone Marrow Transplant 2003; 32:915-923.

Fraiser LH, Kanekal S, Kehrer JP. Cyclophosphamide toxicity. Characterising and avoiding the problem. Drugs 1991;42:781-795.

Freedman AS, Gribben JG, Neuberg D, et al. High-dose therapy and autologous bone marrow transplantation in patients with follicular lymphoma during first remission. Blood 1996;88:2780-2786.

Freytes CO, Loberiza FR, Rizzo JD, et al. Myeloablative allogeneic hematopoietic stem cell transplantation in patients who experience relapse after autologous stem cell transplantation for lymphoma: a report of the International Bone Marrow Transplant Registry. Blood 2004;104:3797-3803.

Fruehauf S, Haas R, Conradt C, et al. Peripheral blood progenitor cell (PBPC) counts during steady-state hematopoiesis allow to estimate the yield of mobilized PBPC after filgrastim (R-metHuG-CSF)-supported cytotoxic chemotherapy. Blood 1995;85:2619-2626.

Fujimaki K, Maruta A, Yoshida M, et al. Severe cardiac toxicity in hematological stem cell transplantation: predictive value of reduced left ventricular ejection fraction. Bone Marrow Transplant 2001;27:307-310.

Fung HC, Cohen S, Rodriguez R et al. Reduced-intensity allogeneic stem cell transplantation for patients whose prior autologous stem cell transplantation for hematologic malignancy failed. Biol Blood Marrow Transplant 2003;9:649-656.

Gandhi MK, Jestice K, Scott MA, Bloxham D, Bass G, Marcus RE. The minimum CD34 threshold depends on prior chemotherapy in autologous peripheral blood stem cell recipients. Bone Marrow Transplant 1999;23:9-13.

Gardner SF, Lazarus HM, Bednarczyk EM, et al. High-dose cyclophosphamide-induced myocardial damage during BMT: assessment by positron emission tomography. Bone Marrow Transplant 1993a;12:139-144.

Gardner RV, Lerner C, Astle CM, Harrison DE. Assessing permanent damage to primitive hematopoietic stem cells after chemotherapy using the competitive repopulation assay. Cancer Chemother Pharmacol 1993b;32:450-454.

Gazitt Y, Callander N, Freytes CO, et al. Peripheral blood stem cell mobilization with cyclophosphamide in combination with G-CSF, GM-CSF, or sequential GM-CSF/G-CSF in non-Hodgkin's lymphoma patients: a randomized prospective study. J Hematother Stem Cell Res 2000;9:737-748.

Gazitt Y. Comparison between granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in the mobilization of peripheral blood stem cells. Curr Opin Hematol 2002;9:190-198.

Geisler C, Elonen E, Kolstad A, et al. Nordic mantle cell lymphoma (MCL) project: prolonged follow-up of 86 patients treated with BEAM/BEAC +PBSCT confirm that addition of high-dose AraC and rituximab to CHOP induction + in vivo purging with rituximab increases clinical and molecular response rates, pcr-negative grafts, failure-free and overall survival. Blood 2004;104: (supp l): 6a (abstr).

Gianni AM, Bregni M, Siena S, et al. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. N Engl J Med 1997;336:1290-1297.

Gianni AM, Magni M, Martelli M, et al. Long-term remission in mantle cell lymphoma following high-dose sequential chemotherapy and in vivo rituximab-purged stem cell autografting (R-HDS regimen). Blood 2003;10:749-755.

Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood 2001;97:631-637.

Gisselbrecht C, Gaulard P, Lepage E, et al. Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Blood 1998;92:76-82.

Gisselbrecht C, Lepage E, Molina T, et al. Shortened first-line high-dose chemotherapy for patients with poor-prognosis aggressive lymphoma. J Clin Oncol 2002;20:2472-2479.

Glaspy JA. Economic considerations in the use of peripheral blood progenitor cells to support high-dose chemotherapy. Bone Marrow Transplant 1999;23:21-27.

Goldberg MA, Antin JH, Guinan EC, Rappeport JM. Cyclophosphamide cardiotoxicity: an analysis of dosing as a risk factor. Blood 1986;68:1114-1118.

Goldschmidt H, Hegenbart U, Wallmeier M, Hohaus S, Haas R. Factors influencing collection of peripheral blood progenitor cells following high-dose cyclophosphamide and granulocyte colony-stimulating factor in patients with multiple myeloma. Br J Haematol 1997;98:736-744.

Goldstone AH, Williams CD, Mackinnon S. Role of nonmyeloablative allogeneic stem-cell transplantation after failure of autologous transplantation in patients with lymphoproliferative malignancies. J Clin Oncol 2002;20:4022-4031.

Gottdiener JS, Appelbaum FR, Ferrans VJ, Deisseroth A, Ziegler J. Cardiotoxicity associated with high-dose cyclophosphamide therapy. Arch Intern Med 1981;141:758-763.

Gralow JR, Livingston RB. University of Washington high-dose cyclophosphamide, mitoxantrone, and etoposide experience in metastatic breast cancer: unexpected cardiac toxicity. J Clin Oncol 2001;19:3903-3904.

Gratwohl A, Schmid O, Baldomero H, Horisberger B, Urbano-Ispizua A. Haematopoietic stem cell transplantation (HSCT) in Europe 2002. Changes in indication and impact of team density. A report of the EBMT activity survey. Bone Marrow Transplant 2004;34:855-875.

Grigg AP, Roberts AW, Raunow H, et al. Optimizing dose and scheduling of filgrastim (granulocyte colony-stimulating factor) for mobilization and collection of peripheral blood progenitor cells in normal volunteers. Blood 1995;86:4437-4445.

Grothues F, Smith GC, Moon JC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. Am J Cardiol 2002;90:29-34.

Haas R, Ho AD, Bredthauer U, et al. Successful autologous transplantation of blood stem cells mobilized with recombinant human granulocyte-macrophage colony-stimulating factor. Exp Hematol 1990;18:94-98.

Haas R, Mohle R, Fruhauf S, et al. Patient characteristics associated with successful mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. Blood 1994;83:3787-3794.

Haioun C, Lepage E, Gisselbrecht C, et al. Benefit of autologous bone marrow transplantation over sequential chemotherapy in poor-risk aggressive non-Hodgkin's lymphoma: updated results of the prospective study LNH87-2. J Clin Oncol 1997;15:1131-1137.

Haioun C, Lepage E, Gisselbrecht C, et al. Survival benefit of high-dose therapy in poor-risk aggressive non-Hodgkin's lymphoma: final analysis of the prospective LNH87-2 protocol--a groupe d'Etude des lymphomes de l'Adulte study. J Clin Oncol 2000;18:3025-3030.

Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994;84:1361-1392.

Harris NL, Jaffe ES, Diebold J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting Airlie House, Virginia, November 1997. J Clin Oncol 1999;17:3835-3849.

Hartikainen JE, Malik M, Staunton A, Poloniecki J, Camm AJ. Distinction between arrhythmic and nonarrhythmic death after acute myocardial infarction based on heart rate variability, signal averaged electrocardiogram, ventricular arrhythmias and left ventricular ejection fraction. J Am Coll Cardiol 1996;28:296-304.

Hertenstein B, Stefanic M, Schmeiser T, et al. Cardiac toxicity of bone marrow transplantation: predictive value of cardiologic evaluation before transplant. J Clin Oncol 1994;12:998-1004.

Hidalgo JD, Krone R, Rich MW, et al. Supraventricular tachyarrhythmias after hematopoietic stem cell transplantation: incidence, risk factors and outcomes. Bone Marrow Transplant 2004;34:615-619.

Hiddemann W, Buske C, Dreyling M, et al. Treatment strategies in follicular lymphomas: current status and future perspectives. J Clin Oncol 2005;23:6394-6399.

Horning SJ, Negrin RS, Chao JC, Long GD, Hoppe RT, Blume KG. Fractionated total-body irradiation, etoposide, and cyclophosphamide plus autografting in Hodgkin's disease and non-Hodgkin's lymphoma. J Clin Oncol 1994;12:2552-2258.

Howe R, Micallef IN, Inwards DJ, et al. Secondary myelodysplastic syndrome and acute myelogenous leukemia are significant complications following autologous stem cell transplantation for lymphoma. Bone Marrow Transplant 2003;32:317-324.

Ikaheimo MJ, Niemelä KO, Linnaluoto MM, Jakobsson MJ, Takkunen JT, Taskinen PJ. Early cardiac changes related to radiation therapy. Am J Cardiol 1985;56:943-946.

Jantunen E, Wiklund T, Juvonen E, et al. Autologous stem cell transplantation in adult patients with peripheral T-cell lymphoma: a nation-wide survey. Bone Marrow Transplant 2004;33:405-410.

Jantunen E, Itälä M, Lehtinen T, et al. Early treatment-related mortality in adult autologous stem cell transplant recipients: a nation-wide survey of 1482 transplanted patients. Eur J Haematol 2006;76:245-250.

Johnsen HE, Bjorkstrand B, Carlson K, et al. Outcome for patients with leukemia, multiple myeloma and lymphoma who relapse after high dose therapy and autologous stem cell support. Leuk Lymphoma 1996;24:81-91.

Kaiser U, Uebelacker I, Abel U, et al. Randomized study to evaluate the use of high-dose therapy as part of primary treatment for "aggressive" lymphoma. J Clin Oncol 2002;20:4413-4419.

Kakavas PW, Ghalie R, Parrillo JE, Kaizer H, Barron JT. Angiotensin converting enzyme inhibitors in bone marrow transplant recipients with depressed left ventricular function. Bone Marrow Transplant 1995;15:859-861.

Katayama Y, Battista M, Kao W-M, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell 2006;124:407-421.

Katz J, Milliken MC, Stray-Gundersen J, et al. Estimation of human myocardial mass with MR imaging. Radiology 1988;169:495-498.

Kessinger A, Armitage JO, Landmark JD, Weisenburger DD. Reconstitution of human hematopoietic function with autologous cryopreserved circulating stem cells. Exp Hematol 1986;14:192-196.

Kessinger A, Bishop MR, Jackson JD, et al. Erythropoietin for mobilization of circulating progenitor cells in patients with previously treated relapsed malignancies. Exp Hematol 1995;23:609-612.

Kessinger A, Sharp JG. The whys and hows of hematopoietic progenitor and stem cell mobilization. Bone Marrow Transplant 2003;31:319-329.

Ketterer N, Salles G, Moullet I, et al. Factors associated with successful mobilization of peripheral blood progenitor cells in 200 patients with lymphoid malignancies. Br J Haematol 1998;103:235-242.

Ketterer N, Sonet A, Dumontet C, et al. Toxicities after peripheral blood progenitor cell transplantation for lymphoid malignancies: analysis of 300 cases in a single institution. Bone Marrow Transplant 1999;23:1309-1315.

Keung Y-K, Lau S, Elkayam U, Chen S-C, Douer D. Cardiac arrhythmia after infusion of cryopreserved stem cells. Bone Marrow Transplant 1994;14:363-367.

Kewalramani T, Zelenetz AD, Hedrick EE, et al. High-dose chemoradiotherapy and autologous stem cell transplantation for patients with primary refractory aggressive non-Hodgkin lymphoma: an intention-to-treat analysis. Blood 2000;96:2399-2404.

Kewalramani T, Nimer SD, Zelenetz AD, et al. Progressive disease following autologous transplantation in patients with chemosensitive relapsed or primary refractory Hodgkin's disease or aggressive non-Hodgkin's lymphoma. Bone Marrow Transplant 2003;32:673-679.

Khouri IF, Lee MS, Romaguera J, et al. Allogeneic hematopoietic transplantation for mantle-cell lymphoma: molecular remissions and evidence of graft-versus-malignancy. Ann Oncol 1999;10: 1293-1299.

Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 1987;59:256-262.

Kluin-Nelemans HC, Zagonel V, Anastasopoulou A, et al. Standard chemotherapy with or without high-dose chemotherapy for aggressive non-Hodgkin's lymphoma: randomized phase III EORTC study. J Natl Cancer Inst 2001;9:22-30.

Kobbe G, Sohngen D, Bauser U, et al. Factors influencing G-CSF-mediated mobilization of hematopoietic progenitor cells during steady-state hematopoiesis in patients with malignant lymphoma and multiple myeloma. Ann Hematol 1999;78:456-462.

Koc ON, Gerson SL, Cooper BW, et al. Randomized cross-over trial of progenitor-cell mobilization: high-dose cyclophosphamide plus granulocyte colony-stimulating factor (G-CSF) versus granulocyte-macrophage colony-stimulating factor plus G-CSF. J Clin Oncol 2000;18:1824-30.

Kornblau S, Benson AB, Catalano R, et al. Management of cancer treatment-related diarrhea. Issues and therapeutic strategies. J Pain Symptom Manage 2000;19:118-129.

Kotasek D, Shepherd KM, Sage RE, et al. Factors affecting blood stem cell collections following high-dose cyclophosphamide mobilization in lymphoma, myeloma and solid tumors. Bone Marrow Transplant 1992;9:11-17.

Kremer LC, van der Pal HJ, Offringa M, van Dalen EC, Voute PA. Frequency and risk factors of subclinical cardiotoxicity after anthracycline therapy in children: a systematic review. Ann Oncol 2002;13:819-829.

Krieger MS, Schiller G, Berenson JR, et al. Collection of peripheral blood progenitor cells (PBPC) based on a rising WBC and platelet count significantly increases the number of CD34+ cells. Bone Marrow Transplant 1999;24:25-28.

Krishnaswamy P, Lubien E, Clopton P, et al. Utility of B-natriuretic peptide levels in identifying patients with left ventricular systolic or diastolic dysfunction. Am J Med 2001;111:274-279.

Kupari M, Volin L, Suokas A, Timonen T, Hekali P, Ruutu T. Cardiac involvement in bone marrow transplantation: electrocardiographic changes, arrhythmias, heart failure and autopsy findings. Bone Marrow Transplant 1990a;5:91-98.

Kupari M, Volin L, Suokas A, Hekali P, Ruutu T. Cardiac involvement in bone marrow transplantation: serial changes in left ventricular size, mass and performance. J Intern Med 1990b;227:259-266.

Ladetto M, Corradini P, Vallet S, et al. High rate of clinical and molecular remissions in follicular lymphoma patients receiving high-dose sequential chemotherapy and autografting at diagnosis: a multicenter, prospective study by the Gruppo Italiano Trapianto Midollo Osseo (GITMO). Blood 2002;100:1559-1565.

Lapidot T, Petit I. Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. Exp Hematol 2002;30:973-981.

Laszlo D, Galieni P, Raspadori D, et al. Fludarabine containing-regimens may adversely affect peripheral blood stem cell collection in low-grade non Hodgkin lymphoma patients. Leuk Lymphoma 2000; 37:157-161.

Lebsack ME, Lange M, Garrison L. Granulocyte-macrophage colony-stimulating factor/interleukin-3 fusion protein versus granulocyte-macrophage colony-stimulating factor after autologous bone marrow transplantation for non-Hodgkin's lymphoma: results of a randomized double-blind trial. J Clin Oncol 1997;15:1617-1623.

Lee CK, Harman GS, Hohl RJ, Gingrich RD. Fatal cyclophosphamide cardiomyopathy: its clinical course and treatment. Bone Marrow Transplant 1996;18:573-577.

Lee JL, Gooley T, Bensinger W, Schiffman K, McDonald GB. Veno-occlusive disease of the liver after busulfan, melphalan, and thiotepa conditioning therapy: incidence, risk factors, and outcome. Biol Blood Marrow Transplant 1999;5:306-315.

Lehmann S, Isberg B, Ljungman P, Paul C. Cardiac systolic function before and after hematopoietic stem cell transplantation. Bone Marrow Transplant 2000;26:187-192.

Lele SS, Durrant ST, Atherton JJ, et al. Demonstration of late cardiotoxicity following bone marrow transplantation by assessment of exercise diastolic filling characteristics. Bone Marrow Transplant 1996;17:1113-1118.

Lemoli RM, de Vivo A, Damiani D, et al. Autologous transplantation of granulocyte colony-stimulating factor-primed bone marrow is effective in supporting myeloablative chemotherapy in patients with hematologic malignancies and poor peripheral blood stem cell mobilization. Blood 2003;102:1595-1600.

Lenain C, Dumontet C, Gargi T, et al. Second autologous transplantation after failure of a first autologous transplant in 18 patients with non-Hodgkin's lymphoma. Hematol J 2004;5:403-409.

Lenihan D and Bashore T. Left ventricular diastolic function. In: Gerson M ed. Cardiac Nuclear Medicine, 3rd ed. New York, NY: McGraw-Hill; 1997:399-413.

Lenz G, Dreyling M, Schiegnitz E, et al. Myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission prolongs progression-free survival in follicular lymphoma: results of a prospective, randomized trial of the German Low-Grade Lymphoma Study Group. Blood 2004;104:2667-74.

Lerman A, Gibbons RJ, Rodeheffer RJ, et al. Circulating N-terminal atrial natriuretic peptide as a marker for symptomless left-ventricular dysfunction. Lancet 1993;341:1105-1109.

Levine ES, Friedman HS, Griffith OW, Colvin OM, Raynor JH, Lieberman M. Cardiac cell toxicity induced by 4-hydroperoxycyclophosphamide is modulated by glutathione. Cardiovasc Res 1993; 27:1248-1253.

Lim T, Ashrafian H, Dwivedi G, Collinson P, Senior R. Increased left atrial volume index is an independent predictor of raised serum natriuretic peptide in patients with suspected heart failure but normal left ventricular ejection fraction: implication for diagnosis of diastolic heart failure. Eur Heart J 2006;8: 38-45.

Lipshultz SE, Sallan SE. Cardiovascular abnormalities in long-term survivors of childhood malignancy. J Clin Oncol 1993;11:1199-1203.

Lopez-Guillermo A, Montserrat E, Bosch F, Terol MJ, Campo E, Rozman C. Applicability of the International Index for aggressive lymphomas to patients with low-grade lymphoma. J Clin Oncol 1994;12:1343-1348.

Lopez-Guillermo A, Cid J, Salar A, et al. Peripheral T-cell lymphomas: initial features, natural history, and prognostic factors in a series of 174 patients diagnosed according to the R.E.A.L. Classification. Ann Oncol 1998;9:849-855.

Lyman GH. Guidelines of the National Comprehensive Cancer Network on the use of myeloid growth factors with cancer chemotherapy: a review of the evidence. J Natl Compr Canc Netw 2005;3:557-571.

Magga J, Vuolteenaho O, Tokola H, Marttila M, Ruskoaho H. B-type natriuretic peptide: a myocyte-specific marker for characterizing load-induced alterations in cardiac gene expression. Ann Med 1998;1:39-45.

Maisel A. B-type natriuretic peptide levels: a potential novel "white count" for congestive heart failure. J Card Fail 2001;7:183-193.

Maloney DG, Liles TM, Czerwinski DK, et al. Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. Blood 1994;84:2457-2466.

Maloney DG. Graft-vs.-lymphoma effect in various histologies of non-Hodgkin's lymphoma. Leuk Lymphoma 2003;3:99-105.

Marcus R, Imrie K, Belch A, et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. Blood 2005;105:1417-1423.

Martin-Murea S, Voso MT, Hohaus S, et al. The dose of granulocyte colony-stimulating factor administered following cytotoxic chemotherapy is not related to the rebound level of circulating CD34+ haemopoietic progenitor cells during marrow recovery. Br J Haematol 1998;101:582-585.

McDonald GB, Slattery JT, Bouvier ME et al. Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. Blood 2003;101:2043-2048.

McLaughlin P, Grillo-Lopez AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998;16:2825-2833.

McQuaker I, Haynes A, Stainer C, Byrne J, Russell N. Mobilisation of peripheral blood stem cells with IVE and G-CSF improves CD34+ cell yields and engraftment in patients with non-Hodgkin's lymphomas and Hodgkin's disease. Bone Marrow Transplant 1999;24:715-722.

Meinardi MT, Van Der Graaf WT, Gietema JA, et al. Evaluation of long term cardiotoxicity after epirubicin containing adjuvant chemotherapy and locoregional radiotherapy for breast cancer using various detection techniques. Heart 2002;88:81-82.

Micallef IN, Apostolidis J, Rohatiner AZ, et al. Factors which predict unsuccessful mobilisation of peripheral blood progenitor cells following G-CSF alone in patients with non-Hodgkin's lymphoma. Hematol J 2000;1:367-73.

Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981;47:207-214.

Miller T, Grossman S, Schectman K, Biello D, Ludbrook P, Ehsani A. Left ventricular diastolic filling and its association with age. Am J Cardiol 1986;58:531-535.

Milpied N, Deconinck E, Gaillard F, et al. Initial treatment of aggressive lymphoma with high-dose chemotherapy and autologous stem-cell support. N Engl J Med 2004;350:1287-1295.

Morandi P, Ruffini PA, Benvenuto GM, La VL, Mezzena G, Raimondi R. Serum cardiac troponin I levels and ECG/Echo monitoring in breast cancer patients undergoing high-dose (7 g/m^2) cyclophosphamide. Bone Marrow Transplant 2001;28:277-282.

Morandi P, Ruffini PA, Benvenuto GM, Raimondi R, Fosser V. Cardiac toxicity of high-dose chemotherapy. Bone Marrow Transplant 2005;35:323-34.

Moreau P, Milpied N, Mahe B, et al. Melphalan 220 mg/m^2 followed by peripheral blood stem cell transplantation in 27 patients with advanced multiple myeloma. Bone Marrow Transplant 1999; 23:1003-1006.

Moskowitz CH, Bertino JR, Glassman JR, et al. Ifosfamide, carboplatin, and etoposide: a highly effective cytoreduction and peripheral-blood progenitor-cell mobilization regimen for transplant-eligible patients with non-Hodgkin's lymphoma. J Clin Oncol 1999;17:3776-3785.

Moskowitz CH, Glassman JR, Wuest D, et al. Factors affecting mobilization of peripheral blood progenitor cells in patients with lymphoma. Clin Cancer Res 1998;4:311-316.

Mounier N, Morel P, Haioun C, et al. A multivariate analysis of the survival of patients with aggressive lymphoma: variations in the predictive value of prognostic factors during the course of the disease. Cancer 1998;82:1952-1962.

Mounier N, Gisselbrecht C. Conditioning regimens before transplantation in patients with aggressive non-Hodgkin's lymphoma. Ann Oncol 1998;9:15-21.

Murdych T, Weisdorf DJ. Serious cardiac complications during bone marrow transplantation at the University of Minnesota, 1977-1997. Bone Marrow Transplant 2001;28:283-287.

Nakamae H, Tsumura K, Hino M, Hayashi T, Tatsumi N. QT dispersion as a predictor of acute heart failure after high-dose cyclophosphamide. Lancet 2000;355:805-806.

Narayanasami U, Kanteti R, Morelli J, et al. Randomized trial of filgrastim versus chemotherapy and filgrastim mobilization of hematopoietic progenitor cells for rescue in autologous transplantation. Blood 2001;98:2059-2064.

Neben S, Hellman S, Montgomery M, Ferrara J, Mauch P. Hematopoietic stem cell deficit of transplanted bone marrow previously exposed to cytotoxic agents. Exp Hematol 1993;21:156-162.

Ng R, Green MD. Pegfilgrastim: evidence in support of its use with cytotoxic chemotherapy. Expert Rev Anticancer Ther 2005;5:585-590.

Nishimura R, Tajik A. Evaluation of diastolic filling of left ventricle in health and disease: Doppler echogardiography in the clinician's Rosetta Stone. J Am Coll Cardiol 1997;30:8-18.

Niwa N, Watanabe E, Hamaguchi M, et al. Early and late elevation of plasma atrial and brain natriuretic peptides in patients after bone marrow transplantation. Ann Hematol 2001;80:460-465.

Nousiainen T, Jantunen E, Vanninen E, Remes J, Vuolteenaho O, Hartikainen J. Natriuretic peptides as markers of cardiotoxicity during doxirubicin treatment for non-Hodgkin's lymphoma. Eur J Haematol 1999;62:135-141.

Nousiainen T, Vanninen E, Jantunen E, et al. Neuroendocrine changes during the evolution of doxorubicin –induced left ventricular dysfunction in adult lymphoma patients. Clin Sci 2001;101:601-607.

Orazi A, Cattoretti G, Schiro R, et al. Recombinant human interleukin-3 and recombinant human granulocyte-macrophage colony stimulating factor administered in vivo after high-dose cyclophosphamide cancer chemotherapy: effect on hematopoiesis and microenvironment in human bone marrow. Blood 1992;79:2610-2619.

Ortega JJ, Olive T, de Heredia CD, Llort A. Secondary malignancies and quality of life after stem cell transplantation. Bone Marrow Transplant 2005;35:83-87.

Otto CM. Textbook of clinical echocardiography. Second edition, W.B. Saunders Ltd, Philadelphia 2002:100-153.

Ozer H. Chemotherapy-induced neutropenia: new approaches to an old problem. Semin Oncol 2003;30:1-2.

Paltiel O, Rubinstein C, Or R, et al. Factors associated with survival in patients with progressive disease following autologous transplant for lymphoma. Bone Marrow Transplant 2003;31:565-569.

Pan D, Moskowitz CH, Zelenetz AD, et al. Rituximab for aggressive non-Hodgkin's lymphomas relapsing after or refractory to autologous stem cell transplantation. Cancer J 2002;8:371-376.

Pandey K. Biology of natriuretic peptides and their receptors. Peptides 2005;26:901-932.

Parillo J. Pathogenetic mechanisms of septic shock. N Engl J Med 1993;328:1471-1477.

Pavone V, Gaudio F, Guarini A, et al. Mobilization of peripheral blood stem cells with high-dose cyclophosphamide or the DHAP regimen plus G-CSF in non-Hodgkin's lymphoma. Bone Marrow Transplant 2002;29:285-290.

Pecego R, Hill R, Appelbaum FR, et al. Interstitial pneumonitis following autologous bone marrow transplantation. Transplantation 1986;42:515-517.

Pelus LM, Horowitz D, Cooper SC, King AG. Peripheral blood stem cell mobilization. A role for CXC chemokines. Crit Rev Oncol Hematol 2002;43:257-275.

Pelus LM, Bian H, King AG, Fukuda S. Neutrophil-derived MMP-9 mediates synergistic mobilization of hematopoietic stem and progenitor cells by the combination of G-CSF and the chemokines GRObeta/CXCL2 and GRObeta/CXCL2delta4. Blood 2004;103:110-119.

Penttilä K, Koukkunen H, Kemppainen A, et al. Myoglobin, creatine kinase MB, troponin T, and troponin I - rapid bedside assays in patients with acute chest pain. Int J Clin Lab Res 1999;29:93-101.

Perkiömäki JS, Zareba W, Nomura A, Andrews M, Kaufman ES, Moss AJ. Repolarization dynamics in patients with long QT syndrome. J Cardiovasc Electrophysiol 2002;13:651-656.

Perry AR, Watts MJ, Peniket AJ, Goldstone AH, Linch DC. Progenitor cell yields are frequently poor in patients with histologically indolent lymphomas especially when mobilized within 6 months of previous chemotherapy. Bone Marrow Transplant 1998;21:1201-1205.

Petit I, Szyper-Kravitz M, Nagler A, et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immunol 2002;3:687-694.

Petros WP, Broadwater G, Berry D, et al. Association of high-dose cyclophosphamide, cisplatin, and carmustine pharmacokinetics with survival, toxicity, and dosing weight in patients with primary breast cancer. Clin Cancer Res 2002;8:698-705.

Pettengell R, Luft T, de Wynter E, et al. Effects of interleukin-6 on mobilization of primitive haemopoietic cells into the circulation. Br J Haematol 1995;89:237-242.

Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med 1995;333:1540-1545.

Phillips GL, Abboud CN, Bernstein SH, et al. Phase I study for poor-prognosis lymphoma: augmentation of the "BEAM" regimen with escalating dose melphalan using amifostine cytoprotection and autologous hematopoietic stem cell transplantation-a preliminary report. Semin Oncol 2004;31:59-61.

Pihkala J, Saarinen UM, Lundstrom U, et al. Effects of bone marrow transplantation on myocardial function in children. Bone Marrow Transplant 1994;13:149-155.

Postma A, Bink-Boelkens MT, Beaufort-Krol GC, et al. Late cardiotoxicity after treatment for a malignant bone tumor. Med Pediatr Oncol 1996;26:230-237.

Quinones M. Assessment of diastolic function. Progress Cardiovasc Dis 2005;47:340-355.

Rapoport AP, Miller Watelet LF, Linder T, et al. Analysis of factors that correlate with mucositis in recipients of autologous and allogeneic stem-cell transplants. J Clin Oncol 1999;17:2446-2453.

Redfield M, Rodeheffer R, Jacobsen S, Mahoney D, Bailey K, Burnett Jr J. Plasma brain natriuretic peptide concentration: impact of age and gender. J Am Coll Cardiol 2002;40:976-982.

Reimer P, Schertlin T, Rudiger T, et al. Myeloablative radiochemotherapy followed by autologous peripheral blood stem cell transplantation as first-line therapy in peripheral T-cell lymphomas: first results of a prospective multicenter study. Hematol J 2004;5:304-311.

Remes K, Matinlauri I, Grenman S, et al. Daily measurements of blood CD34+ cells after stem cell mobilization predict stem cell yield and posttransplant hematopoietic recovery. J Hematother 1997;6:13-19.

Richardson P, Murakami C, Jin, Z, et al. Multi-institutional use of defibrotide in 88 patients after stem cell transplantation with severe veno-occlusive disease and multisystem organ failure: response without significant toxicity in a high-risk population and factors predictive of outcome. Blood 2002;100:4337-4343.

Ritchie JL, Bateman TM, Bonow RO, et al. Guidelines for clinical use of cardiac radionuclide imaging. J Nucl Cardiol 1995;2:172-192.

Roberts AW, DeLuca E, Begley CG, Basser R, Grigg AP, Metcalf D. Broad inter-individual variations in circulating progenitor cell numbers induced by granulocyte colony-stimulating factor therapy. Stem Cells 1995;13:512-516.

Rodriguez J, Munsell M, Yazji S, et al. Impact of high-dose chemotherapy on peripheral T-cell lymphomas. J Clin Oncol 2001;19:3766-3770.

Rose M, Lee FA, Gollerkeri A, et al. The feasibility of high-dose chemotherapy in breast cancer patients with impaired left ventricular function. Bone Marrow Transplant 2000;26:133-139.

Rosenwald A, Wright G, Wiestner A, et al. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. Cancer Cell 2003;3:185-197.

Rowlings PA, Bayly JL, Rawling CM, Juttner CA, To LB. A comparison of peripheral blood stem cell mobilisation after chemotherapy with cyclophosphamide as a single agent in doses of 4 g/m^2 or 7 g/m^2 in patients with advanced cancer. Aust N Z J Med 1992;22:660-664.

Ruskoaho H. Cardiac hormones as diagnostic tools in heart failure. Endocr Rev 2003;24:341-456.

Russell NH, McQuaker G, Stainer C, Byrne JL, Haynes AP. Stem cell mobilisation in lymphoproliferative diseases. Bone Marrow Transplant 1998;22:935-940.

Ruutu T, Eriksson B, Remes K, et al. Nordic Bone Marrow Transplantation Group. Ursodeoxycholic acid for the prevention of hepatic complications in allogeneic stem cell transplantation. Blood 2002; 100:1977-1983.

Sagnella G. Measurement and significance of circulating natriuretic peptides in cardiovascular disease. Clin Sci 1998;95:519-529.

Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978;58:1072-1083.

Sakata-Yanagimoto M, Kanda Y, Nakagawa M, et al. Predictors for severe cardiac complications after hematopoietic stem cell transplantation. Bone Marrow Transplant 2004;33:1043-1047.

Salar A, Sierra J, Gandarillas M, Caballero MD, et al. GEL/TAMO Spanish Cooperative Group. Autologous stem cell transplantation for clinically aggressive non-Hodgkin's lymphoma: the role of preparative regimens. Bone Marrow Transplant 2001;27:405-412.

Salton CJ, Chuang ML, O'Donnell CJ, et al. Gender differences and normal left ventricular anatomy in an adult population free of hypertension. A cardiovascular magnetic resonance study of the Framingham Heart Study offspring cohort. J Am Coll Cardiol 2002;39:1055-1060.

Samuels BL, Bitran JD. High-dose intravenous melphalan. J Clin Oncol 1995;13:1786-1799.

Sandri MT, Salvatici M, Cardinale D, et al. N-terminal pro-B-type natriuretic peptide after high-dose chemotherapy: a marker predictive of cardiac dysfunction? Clin Chem 2005;51:1405-1410.

Sato T, Laver JH, Ogawa M. Reversible expression of CD34 by murine hematopoietic stem cells. Blood 1999;94:2548-2554.

Schmitz N, Linch DC, Dreger P, et al. Randomised trial of filgrastim-mobilised peripheral blood progenitor cell transplantation versus autologous bone-marrow transplantation in lymphoma patients. Lancet 1996;347:353-357.

Schmitz N, Ljungman P, Cordonnier C, et al. Lenograstim after autologous peripheral blood progenitor cell transplantation: results of a double-blind, randomized trial. Bone Marrow Transplant 2004;34:955-962.

Schouten HC, Qian W, Kvaloy S, et al. High-dose therapy improves progression-free survival and survival in relapsed follicular non-Hodgkin's lymphoma: results from the randomized European CUP trial. J Clin Oncol 2003;21:3918-3927.

Schrama JG, Holtkamp MJ, Baars JW, Schornagel JH, Rodenhuis S. Toxicity of the high-dose chemotherapy CTC regimen (cyclophosphamide, thiotepa, carboplatin): the Netherlands Cancer Institute experience. Br J Cancer 2003;88:1831-1838.

Seggewiss R, Buss EC, Herrmann D, Goldschmidt H, Ho AD, Fruehauf S. Kinetics of peripheral blood stem cell mobilization following G-CSF-supported chemotherapy. Stem Cells 2003;21:568-574.

Shipp MA, Neuberg D, Janicek M, Canellos GP, Shulman LN. High-dose CHOP as initial therapy for patients with poor-prognosis aggressive non-Hodgkin's lymphoma: a dose-finding pilot study. J Clin Oncol 1995;13:2916-2923.

Simon M, Hahn T, Ford LA, et al. Retrospective multivariate analysis of hepatic veno-occlusive disease after blood or marrow transplantation: possible beneficial use of low molecular weight heparin. Bone Marrow Transplant 2001;27:627-633.

Snowden JA, Hill GR, Hunt P, et al. Assessment of cardiotoxicity during haemopoietic stem cell transplantation with plasma brain natriuretic peptide. Bone Marrow Transplant 2000;26:309-313.

Solal-Celigny P, Roy P, Colombat P, et al. Follicular lymphoma international prognostic index. Blood 2004;104:1258-1265.

Song KW, Mollee P, Keating A, Crump M. Autologous stem cell transplant for relapsed and refractory peripheral T-cell lymphoma: variable outcome according to pathological subtype. Br J Haematol 2003;120:978-985.

Spielberger R, Stiff P, Bensinger W, et al. Palifermin for oral mucositis after intensive therapy for hematologic cancers. N Engl J Med 2004;351:2590-2598.

Spriano M, Clavio M, Carrara P, et al. Fludarabine in untreated and previously treated B-CLL patients: a report on efficacy and toxicity. Haematologica 1994;79:218-224.

Stanton E, Hansen M, Wijeysundera HC, Kupchak P, Hall C, Rouleau JL. A direct comparison of the natriuretic peptides and their relationship to survival in chronic heart failure of a presumed non-ischaemic origin. Eur J Heart Fail 2005;7:557-565.

Staudt LM. Molecular diagnosis of the hematologic cancers. N Engl J Med 2003;348:1777-1785.

Steinherz LJ, Steinherz PG, Mangiacasale D, et al. Cardiac changes with cyclophosphamide. Med Pediatr Oncol 1981;9:417-422.

Steinherz LJ, Graham T, Hurwitz R, et al. Guidelines for cardiac monitoring of children during and after anthracycline therapy: report of the Cardiology Committee of the Childrens Cancer Study Group. Pediatrics 1992;89:942-949.

Stiff PJ, Koester AR, Eagleton LE, Hindman T, Braud E, Weidner MK. Autologous stem cell transplantation using peripheral blood stem cells. Transplantation 1987;44:585-588.

Stiff PJ. Management strategies for the hard-to-mobilize patient. Bone Marrow Transplant 1999;23:29-33.

Stockerl-Goldstein KE, Reddy SA, Horning SF, et al. Favorable treatment outcome in non-Hodgkin's lymphoma patients with "poor" mobilization of peripheral blood progenitor cells. Biol Blood Marrow Transplant 2000;6:506-512.

Styler M, Topolsky D, Crilley P, et al. Transient high grade heart block following autologous bone marrow infusion. Bone Marrow Transplant 1992;10:435-438.

Sugrue MW, Williams K, Pollock BH, et al. Characterization and outcome of "hard to mobilize'" lymphoma patients undergoing autologous stem cell transplantation. Leuk Lymphoma 2000;39:509-519.

Swedberg K, Cleland J, Dargie H, et al. Guidelines for the diagnosis and treatment of chronic heart failure: the task force for the diagnosis and treatment of chronic heart failure of the European Society of Cardiology. Eur Heart J 2005;26:1115-1140.

Tarella C, Castellino C, Locatelli F, et al. G-CSF administration following peripheral blood progenitor cell (PBPC) autograft in lymphoid malignancies: evidence for clinical benefits and reduction of treatment costs. Bone Marrow Transplant 1998;21:401-407.

Tarella C, Zallio F, Caracciolo D, et al. Hemopoietic progenitor cell mobilization and harvest following an intensive chemotherapy debulking in indolent lymphoma patients. Stem Cells 1999;17:55-61.

The Non-Hodgkin's Lymphoma Classification Project. Effect of age on the characteristics and clinical behavior of non-Hodgkin's lymphoma patients. Ann Oncol 1997;8:973-978.

To LB, Haylock DN, Simmons PJ, Juttner CA. The biology and clinical uses of blood stem cells. Blood 1997;89:2233-2258.

To LB, Shepherd KM, Lam-Po-Tang R, Szer J, McGrath KM. Guidelines for the collection, processing, storage and of administration of hemopoietic stem and progenitor cells for transplantation. Pathology 1998;30:276-285.

To LB, Bashford J, Durrant S, et al. Successful mobilization of peripheral blood stem cells after addition of ancestim (stem cell factor) in patients who had failed a prior mobilization with filgrastim (granulocyte colony-stimulating factor) alone or with chemotherapy plus filgrastim. Bone Marrow Transplant 2003;31:371-378.

Tournilhac O, Cazin B, Lepretre S, et al. Impact of frontline fludarabine and cyclophosphamide combined treatment on peripheral blood stem cell mobilization in B-cell chronic lymphocytic leukemia. Blood 2004;103:363-365.

Vaickus L, Letendre L. Pericarditis induced by high-dose cytarabine therapy. Arch Intern Med 1984;144:1868-1869.

Vaishampayan U, Karanes C, Du W, Varterasian M, al-Katib A. Outcome of relapsed non-Hodgkin's lymphoma patients after allogeneic and autologous transplantation. Cancer Invest 2002;20:303-310.

Valteau-Couanet D, Faucher C, Auperin A, et al. Cost effectiveness of day 5 G-CSF (Lenograstim ((R))) administration after PBSC transplantation: results of a SFGM-TC randomised trial. Bone Marrow Transplant 2005;36:547-552.

van Besien K, Tabocoff J, Rodriguez M, et al. High-dose chemotherapy with BEAC regimen and autologous bone marrow transplantation for intermediate grade and immunoblastic lymphoma: durable complete remissions, but a high rate of regimen-related toxicity. Bone Marrow Transplant 1995;15:549-555.

van Besien K, Loberiza FR Jr, Bajorunaite R, et al. Comparison of autologous and allogeneic hematopoietic stem cell transplantation for follicular lymphoma. Blood 2003;102:3521-3529.

Vandenberghe E, Ruiz de EC, Loberiza FR, et al. Outcome of autologous transplantation for mantle cell lymphoma: a study by the European Blood and Bone Marrow Transplant and Autologous Blood and Marrow Transplant Registries. Br J Haematol 2003;120:793-800.

Vantelon JM, Koscielny S, Brault P, et al. Scoring system for the prediction of successful peripheral blood stem cell (PBSC) collection in non-Hodgkin's lymphoma (NHL): application in clinical practice. Bone Marrow Transplant 2000;25:495-499.

Vellenga E, van Agthoven M, Croockewit AJ, et al. Autologous peripheral blood stem cell transplantation in patients with relapsed lymphoma results in accelerated haematopoietic reconstitution, improved quality of life and cost reduction compared with bone marrow transplantation: the Hovon 22 study. Br J Haematol 2001;114:319-326.

Verdonck LF, Dekker AW, de Gast GC, van Kempen ML, Lokhorst HM, Nieuwenhuis HK. Salvage therapy with ProMACE-MOPP followed by intensive chemoradiotherapy and autologous bone marrow transplantation for patients with non-Hodgkin's lymphoma who failed to respond to first-line CHOP. J Clin Oncol 1992;10:1949-1954.

Verdonck LF, van Putten WL, Hagenbeek A, et al. Comparison of CHOP chemotherapy with autologous bone marrow transplantation for slowly responding patients with aggressive non-Hodgkin's lymphoma. N Engl J Med 1995;332:1045-1051.

Vollmar A. The role of atrial natriuretic peptide in the immune system. Peptides 2005;26:1086-1094.

Vose JM, Bierman PJ, Anderson JR, et al. Progressive disease after high-dose therapy and autologous transplantation for lymphoid malignancy: clinical course and patient follow-up. Blood 1992;80:2142-2148.

Vose JM, Zhang MJ, Rowlings PA, et al. Autologous transplantation for diffuse aggressive non-Hodgkin's lymphoma in patients never achieving remission: a report from the Autologous Blood and Marrow Transplant Registry. J Clin Oncol 2001;19:406-413.

Wackers FJ, Berger HJ, Johnstone DE, et al. Multiple gated cardiac blood pool imaging for left ventricular ejection fraction: validation of the technique and assessment of variability. Am J Cardiol 1979;43:1159-1566.

Watts MJ, Sullivan AM, Jamieson E, et al. Progenitor-cell mobilization after low-dose cyclophosphamide and granulocyte colony-stimulating factor: an analysis of progenitor-cell quantity and quality and factors predicting for these parameters in 101 pretreated patients with malignant lymphoma. J Clin Oncol 1997;15:535-546.

Watts MJ, Sullivan AM, Leverett D, et al. Back-up bone marrow is frequently ineffective in patients with poor peripheral-blood stem-cell mobilization. J Clin Oncol 1998;16:1554-1560.

Watts MJ, Ings SJ, Flynn M, Dodds D, Goldstone AH, Linch DC. Remobilization of patients who fail to achieve minimal progenitor thresholds at the first attempt is clinically worthwhile. Br J Haematol 2000; 111:287-291.

Wardley AM, Jayson GC, Swindell R, et al. Prospective evaluation of oral mucositis in patients receiving myeloablative conditioning regimens and haemopoietic progenitor rescue. Br J Haematol 2000;110:292-299.

Weaver CH, Petersen FB, Appelbaum FR, et al. High-dose fractionated total-body irradiation, etoposide, and cyclophosphamide followed by autologous stem-cell support in patients with malignant lymphoma. J Clin Oncol 1994;12:2559-2566.

Weaver CH, Hazelton B, Birch R, et al. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. Blood 1995;86:3961-3969.

Weaver CH, Potz J, Redmond J, et al. Engraftment and outcomes of patients receiving myeloablative therapy followed by autologous peripheral blood stem cells with a low CD34+ cell content. Bone Marrow Transplant 1997;19:1103-1110.

Weaver CH, Birch R, Greco FA, et al. Mobilization and harvesting of peripheral blood stem cells: randomized evaluations of different doses of filgrastim. Br J Haematol 1998;100:338-347.

Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. Cell 2000;100:157-168.

Williams CD, Harrison CN, Lister TA, et al. High-dose therapy and autologous stem-cell support for chemosensitive transformed low-grade follicular non-Hodgkin's lymphoma: a case-matched study from the European Bone Marrow Transplant Registry. J Clin Oncol 2001;19:727-735.

Windrum P, Morris TC, Drake MB, Niederwieser D, Ruutu T. Variation in dimethyl sulfoxide use in stem cell transplantation: a survey of EBMT centres. Bone Marrow Transplant 2005;36: 601-603.

Wingard JR, Sostrin MB, Vriesendorp HM, et al. Interstitial pneumonitis following autologous bone marrow transplantation. Transplantation 1988;46:61-65.

Witzig TE. Efficacy and safety of 90Y ibritumomab tiuxetan (Zevalin) radioimmunotherapy for non-Hodgkin's lymphoma. Semin Oncol 2003;30:11-16.

Zangari M, Henzlova MJ, Ahmad S, et al. Predictive value of left ventricular ejection fraction in stem cell transplantation. Bone Marrow Transplant 1999;23:917-920.

Zanjani ED, Almeida-Porada G, Livingston AG, Flake AW, Ogawa M. Human bone marrow CD34- cells engraft in vivo and undergo multilineage expression that includes giving rise to CD34+ cells. Exp Hematol 1998;26:353-360.

Zhong R, Law P, Wong D, Merzouk A, Salari H, Ball ED. Small peptide analogs to stromal derived factor-1 enhance chemotactic migration of human and mouse hematopoietic cells. Exp Hematol 2004;32:470-475.

ORIGINAL PUBLICATIONS
I - VI

Kuopio University Publications D. Medical Sciences

D 360. Huopio, Jukka. Predicting fractures in middle-aged women.
2005. 86 p. Acad. Diss.

D 361. Gül, Mustafa. Cytotoxic and antifungal acetophenone-derived Mannich bases: effects on redox thiols and heat shock proteins.
2005. 68 p. Acad. Diss.

D 362. Virtanen, Jyrki. Homocysteine, folate and cardiovascular diseases.
2005. 65 p. Acad. Diss.

D 363. Tuomainen, Petri. Physical exercise in clinically healthy men and in patients with angiographically documented coronary artery disease with special referense to cardiac autonomic control and warm-up phenomenon.
2005. 125 p. Acad. Diss.

D 364. Lindgren, Annamarja. Cancer incidence in hypertensive patients.
2005. 94 p. Acad. Diss.

D 365. Töyry, Saara. Burnout and self-reported health among Finnish physicians.
2005. 102 p. Acad. Diss.

D 366. Haapalahti, Mila. Nutrition, gastrointestinal food hypersensitivity and functional gastrointestinal disorders in schoolchildren and adolescents.
2005. 612 p. Acad. Diss.

D 367. Lindi, Virpi. Role of the Human PPAR-γ2 Gene on Obesity, Insulin Resistance and Type 2 Diabetes.
2005. 102 p. Acad. Diss.

D 368. Penttilä, Karri. Evaluation of different biochemical methods to detect myocardial injury.
2005. 99 p. Acad. Diss.

D 369. Sipola, Petri. Magnetic resonance imaging in hypertrophic cardiomyopathy.
2005. 160 p. Acad. Diss.

D 370. Pasonen-Seppänen, Sanna. Regulation of keratinocyte differentiation and hyaluronan metabolism in an organotypic keratinocyte culture.
2005. 109 p. Acad. Diss.

D 371. Laukkanen, Jari. Exercise testing in the prediction of cardiovascular diseases and mortality: a prospective population study in men.
2005. 93 p. Acad. Diss.

D 372. Määttä, Sara. Event-related potenital studies on novelty processing and distractibility.
2005. 54 p. Acad. Diss.

D 373. Juutilainen, Auni. Gender, type 2 diabetes and risk of cardiovascular disease.
2005. 54 p. Acad. Diss.

D 374. Purhonen, Sinikka. Prevention of postoperative nausea and vomiting: with special reference to supplemental oxygen, different antiementics and anesthesia regimens.
2006. 85 p. Acad. Diss.

D 375. Tuomainen, Tomi-Pekka. Body iron, atherosclerosis and coronary heart disease.
2006. 85 p. Acad. Diss.