University of Groningen

Long-term chemotherapy-related cardiovascular morbidity

Meinardi, Martin Theodoor

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2001

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Meinardi, M. T. (2001). *Long-term chemotherapy-related cardiovascular morbidity*. [Thesis fully internal (DIV), University of Groningen]. [S.n.].

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

The background of the cover is black, decorated with an abstract pattern of rectangular blocks in various colors including red, yellow, grey, and purple. These blocks are arranged in a way that suggests a staircase or a series of steps, with some blocks appearing to be stacked or offset from each other. The colors are somewhat muted and have a slightly textured appearance.

Long-term Chemotherapy-related Cardiovascular Morbidity

Martin
Meinardi

Long-term chemotherapy-related cardiovascular morbidity

Martin Meinardi

STELLINGEN

bij het proefschrift

"Long-term chemotherapy-related cardiovascular morbidity"

1. De huidige follow-up duur van 10 jaar voor testiscarcinoom patiënten behandeld met cisplatin-bevattende chemotherapie, is voor het opsporen van een recidief voldoende maar voor het opsporen van lange termijn effecten te kort. (dit proefschrift)
2. Chelatie-therapie toegepast in het kader van de behandeling van atherosclerose kan bij testiscarcinoom patiënten, behandeld met cisplatine-bevattende chemotherapie, mogelijk wel van waarde zijn.
3. De mens heeft niet alleen psychisch, maar ook fysiek moeite om eens gekregen edele metalen weer af te staan. (dit proefschrift)
4. Het evalueren van anthracycline cardiotoxiciteit door alleen de systolische functie van het hart in rust te meten, is onvoldoende. (dit proefschrift)
5. Hoge-dosis chemotherapie zoals toegepast bij borstkanker patiënten geeft geen aantoonbare toename van cardiotoxiciteit op korte termijn. (dit proefschrift)
6. Heart rate variability analyse is voor het opsporen van anthracycline cardiotoxiciteit bij de individuele patiënt niet geschikt.
7. Voordat herceptin toegepast kan gaan worden in de adjuvante behandeling van borstkanker dienen de cardiotoxische effecten nauwkeurig te worden geëvalueerd.
8. In de ontwikkeling van kanker zijn omgevingsfactoren van groter belang dan genetische factoren. (N Engl J Med 2000;343:78-85)
9. In de afgelopen decennia is endotheel gepromoveerd van een eenvoudige binnenbekleding van bloedvaten tot het grootste endocriene, paracriene en endocriene orgaan.

10. Een hart zo sterk als dat van een neushoorn, geeft geen garantie dat dit hart een anthracycline kuur kan overleven (Vet Pathol 2000 ;37:86-88)
11. Huwelijksstress verhoogt bij vrouwen de kans op het krijgen van een hartinfarct. Mannen hebben een verhoogde kans op het krijgen van een hartinfarct tijdens het kijken naar voetbal-wedstrijden. Omdat het één met het ander te maken zou kunnen hebben, dient zeker in een matig huwelijk het kijken naar voetbalwedstrijden te worden ontraden. (BMJ 2000;321:1552-54 ; JAMA 2000; 284:3008-14)
12. Om het dreigende tekort aan specialisten op te vangen, dient het budget voor agiogenese te worden vergroot.
13. Doordat de mens zich bindt aan nieuwe communicatie technieken als 'wappen' en 'blue tooth' raakt hij de draad in zijn leven kwijt.
14. Gesteld dat de mens inclusief zijn denkproces niet meer is dan een vat toevallige botsende moleculen, dan impliceert dit dat deze stelling bij toeval is ontstaan en dus geen enkele waarde heeft.

Martin Meinardi, 21 februari 2001

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Meinardi, M.T.

Long-term chemotherapy-related cardiovascular morbidity

Proefschrift Groningen. -Met lit.opg. -Met samenvatting in het Nederlands

ISBN 90-806085-2-1

NUGI 742

© Copyright 2001 M.T. Meinardi

All rights are reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, mechanically, by photocopying, recording or otherwise, without the written permission of the author.

Design and layout: M.T. Meinardi

Printed by: Drukkerij G. van Ark, Haren

RIJKSUNIVERSITEIT GRONINGEN

Long-term chemotherapy-related cardiovascular morbidity

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de Rector Magnificus, Dr. D.F.J. Bosscher,
in het openbaar te verdedigen op
woensdag 21 februari 2001
om 16.00 uur

door

Martin Theodoor Meinardi

geboren op 4 juni 1970
te Groningen

Promotores: Prof. Dr. D.Th. Sleijfer
Prof. Dr. D.J. van Veldhuisen

Co-promotor: Dr. W.T.A. van der Graaf

Referent: Dr. J.A. Gietema

Promotiecommissie: Prof. Dr. H.J.G.M. Crijns
Prof. Dr. Ir. F.E. van Leeuwen
Prof. Dr. N.H. Mulder

Printing costs of this thesis were kindly supported by contributions of the Stichting Werkgroep Interne Oncologie, AstraZeneca BV, Bristol-Myers Squibb BV, Glaxo Wellcome BV, Merck Sharp & Dohme BV, Monsanto Nederland BV (division Searle), Novartis Pharma BV, Pfizer BV, Pharmacia & Upjohn BV, Sanofi Winthrop BV, Servier Nederland BV and Yamanouchi Pharma BV.

CONTENTS

Chapter 1	Introduction	9
Chapter 2	Long-term chemotherapy-related cardiovascular morbidity <i>Cancer Treatment Reviews 2000; 26: 429-47</i>	13
Chapter 3	Detection of anthracycline-induced cardiotoxicity <i>Cancer Treatment Reviews 1999; 25: 237-47</i>	37
Chapter 4	Cardiovascular morbidity in long-term survivors of meta- static testicular cancer <i>Journal of Clinical Oncology 2000; 18: 1725-32</i>	51
Chapter 5	Circulating plasma platinum more than 10 years after cis- platin treatment for testicular cancer <i>The Lancet 2000; 355: 1075-76</i>	65
Chapter 6	Magnesium and phosphorus <i>The Lancet 1998; 352: 1476</i>	69
Chapter 7	Early detection of anthracycline induced cardiotoxicity in asymptomatic patients with normal left ventricular systolic function: autonomic versus echocardiographic variables. <i>Heart 1999; 81: 419-23</i>	71
Chapter 8	Evaluation of long-term cardiotoxicity after epirubicin- containing adjuvant chemotherapy and locoregional radio- therapy for breast cancer, with various detection tech- niques <i>Submitted for publication</i>	81
Chapter 9	Prospective evaluation of early cardiac damage induced by epirubicin-containing adjuvant chemotherapy and locore- gional radiotherapy in breast cancer patients <i>Journal of Clinical Oncology, in press</i>	93
Chapter 10	Future perspectives	107
Chapter 11	Summary	111
	Nederlandse samenvatting	117
References		123
Dankwoord		139

CHAPTER 1

INTRODUCTION

Thanks to the success of chemotherapy in the treatment of curable disseminated neoplasms such as testicular cancer, and the increasing application of neoadjuvant and adjuvant chemotherapy for various tumor types including breast cancer, the number of patients with a normal life expectancy after treatment is rising. Evaluation of possible chronic side-effects of the chemotherapy is therefore becoming increasingly relevant in these patient groups. Investigations with respect to long-term chemotherapy-related cardiovascular morbidity are of particular importance since it may negatively influence the quality of life and the life expectancy of these patients. There is uncertainty concerning this issue since the follow-up of many patients treated with different chemotherapy regimens is limited yet. So far most data on long-term cardiovascular toxicity have been collected in survivors of childhood cancer treated with anthracyclines. Anthracyclines are well-known for their cardiotoxic side-effects. The induction of chronic cardiomyopathy which can culminate in congestive heart failure (CHF) is the one that is most clinically relevant.¹ Most cases of CHF occur within the first year after treatment,² but several studies in long-term survivors of childhood cancer have described patients who developed CHF after a latency of more than 10 years.^{3,4} In addition, subclinical cardiotoxicity has been reported to be present in up to 65% of the survivors, and the prevalence rises with the length of follow-up.^{3,5} Therefore concern exists that also the prevalence of late-onset CHF will increase with longer follow-up. The number of follow-up studies in adults focussed on the long-term cardiovascular toxicity of chemotherapy is limited. This issue is of less relevance in patients with a poor prognosis who will rather die of tumor recurrence than face problems of chronic cardiovascular toxicity.

However, evaluation of chronic cardiovascular toxicity is particularly of importance in patients which can be cured with chemotherapy as is the case with testicular cancer. Most patients with metastatic testicular cancer are young (usually between 20 and 40 years of age) and about 90 % of them can nowadays be cured with cisplatin-based chemotherapy.⁶ Once cured from the tumor these patients are presumed to have a normal life expectancy. This patient cohort is therefore an ideal model to study long-term side effects of chemotherapy. Several chronic side effects of this treatment have been recognized already for a long time, such as nephrotoxicity and neurotoxicity.^{7,8} Furthermore, Raynaud's phenomenon which is considered a manifestation of small vessel disease occurs in about 30 percent.⁹ Moreover, several case reports on myocardial infarctions and cerebrovascular accidents have been reported to occur during or several weeks or months after chemotherapy.¹⁰⁻¹² However, in large follow-up studies no increased risk of cardiovascular disease has been observed so far.^{13,14} On the other hand several studies suggested that these patients develop after treatment an unfavorable cardiovascular risk-profile including hypercholesterolemia and hypertension.¹⁴⁻¹⁶ This cardiovascular risk-profile may have an impact on the development of cardiovascular disease in the long-term.

Another patient group for whom the issue of chronic cardiotoxicity is of importance is the group of breast cancer patients treated with adjuvant chemotherapy. Anthracyclines are nowadays increasingly used in these patients, since they have been shown to result in a better survival compared to regimens without anthracyclines.^{17,18} However, in this group of patients concern exists that in the long-term this survival

benefit is counterbalanced by the risk of CHF caused by these anthracyclines. Evaluation of the cardiac function by means of detection techniques may help to identify patients at risk for CHF in an early subclinical stage of the disease. This may open new ways to medical intervention with for example angiotensin converting enzyme inhibitors with the purpose to delay further deterioration.^{19,20} Most commonly, during and after anthracycline treatment only the systolic function of the heart is evaluated by means of assessment of the left ventricular ejection fraction by radionuclide ventriculography. However, the sensitivity and specificity for early detection of cardiotoxicity has been shown to be limited.²¹ Based on new insights into the pathophysiology of anthracycline cardiotoxicity and the development of CHF in general new and useful detection methods have been proposed that may determine cardiotoxicity in an earlier stage.²²

The aim of this thesis is to evaluate the chronic adverse effects of chemotherapy on the cardiovascular system in survivors of disseminated testicular cancer treated with cisplatin based chemotherapy and in breast cancer patients treated with anthracycline containing adjuvant chemotherapy, with or without high-dose chemotherapy. The studies in survivors of testicular cancer will focus on the prevalence of cardiovascular disease and cardiovascular risk-factors, as well as on other factors that could play a role in the pathogenesis. The studies performed in the breast cancer patients will focus on the determination of the prevalence of (sub)clinical cardiotoxicity and on the evaluation of the usefulness of several detection techniques to determine this cardiotoxicity.

In **chapter 2** an overview of the literature will be presented on the long-term chemotherapy-related cardiovascular toxicity in patients with a good life-expectancy after treatment such as survivors of testicular cancer and malignant lymphomas, and patients treated with adjuvant chemotherapy for breast cancer and colorectal cancer.

In **chapter 3** the literature concerning the detection of anthracycline cardiotoxicity is reviewed. New methods such as the determination of diastolic function parameters, heart rate variability, and natriuretic peptides have been proposed to identify patients at risk for the development of CHF in an early stage.^{23,24} Their value and usefulness in clinical practice are discussed.

Since in previous studies it was shown that a number of testicular cancer patients develops an unfavorable cardiovascular risk-profile after chemotherapy, including hypercholesterolemia and hypertension,^{14,16} concern exists whether these cured patients in the long-term are at an increased risk of developing cardiovascular morbidity and mortality.

In **chapter 4** the results are presented of a follow-up study performed in long-term survivors of testicular cancer who were 10 to 20 years after chemotherapy, focussed on the prevalence of cardiovascular events and cardiovascular risk-factors.

After treatment with cisplatin-based chemotherapy platinum has been demonstrated to retend for years in several organs including the liver.²⁵ Up to 8 years after chemotherapy platinum has been detected in the urine of testicular cancer survivors.²⁶ We investigated whether platinum compounds are still present in the plasma 10-20 years after treatment, and whether this might play a role in the development of chronic cardiovascular toxicity. The results are described in **chapter 5**.

Magnesium deficiency in general has been suggested to play a role in the development of several cardiovascular risk factors including hypertension and dyslipidemia and in the development of cardiovascular events.²⁷⁻²⁹ Cisplatin is known to induce proximal-tubule damage that results in renal magnesium wasting.³⁰ In **chapter 6** we describe whether long-term survivors of testicular cancer treated with cisplatin based chemotherapy still have signs of a disturbed magnesium balance.

The long-term consequences of the incorporation of anthracyclines and related compounds in adjuvant chemotherapy regimens used in high-risk breast cancer with respect to cardiotoxicity have not been established yet. Furthermore the role of high-dose chemotherapy with respect to induction of cardiac dysfunction has not been studied thoroughly. As aforementioned, detection of cardiotoxicity in an early stage may be important since it may identify patients at risk for CHF. We investigated the prevalence of cardiotoxicity in several groups of breast cancer patients, and at the same time we evaluated the usefulness of several detection techniques.

In **chapter 7** the results are presented of an exploratory study on the prevalence of early cardiac damage in breast cancer patients (both locally advanced and metastatic) who had been treated with anthracyclines combined with locoregional irradiation. These patients had all participated in high-dose chemotherapy schedules with autologous bone marrow or peripheral stem cell transplantation. Particularly the prevalence of abnormalities in heart rate variability and diastolic function parameters was investigated.

Subsequently we investigated the prevalence of cardiac damage in a more homogeneous population consisting of patients with locally advanced breast cancer who had been treated 2 or more years previously with anthracycline-containing adjuvant chemotherapy as part of a randomized phase III study. In this study patients were treated with either five cycles of 5-fluorouracil, epirubicin and cyclophosphamide (FEC) or with 4 cycles of FEC followed by high-dose chemotherapy consisting of cyclophosphamide, thiotepa and carboplatin. Cardiac damage was evaluated by determination of the left ventricular ejection fraction by radionuclide ventriculography, echocardiography for evaluation of the diastolic function, heart rate variability analysis calculated from a 24-hr Holter recording, an electrocardiogram and natriuretic peptides. The results are presented in **chapter 8**.

Following this cross-sectional study we performed a prospective evaluation of changes in the cardiac function of other breast cancer patients participating in the same phase III study. The results are described in **chapter 9**. This cardiac evaluation was performed before chemotherapy and at various points of time after chemotherapy by means of the same detection techniques as were used in the cross-sectional study.

After some perspectives for future research described in **chapter 10**, a summary of the results of all investigations is given in **chapter 11**.

CHAPTER 2

Long-term chemotherapy-related cardiovascular morbidity

*M.T. Meinardi¹, J.A. Gietema¹, D.J. van Veldhuisen², W.T.A. van der Graaf¹,
E.G.E. de Vries¹, D. Th. Sleijfer¹*

Departments of Medical Oncology¹ and Cardiology², University Hospital Groningen,
The Netherlands

Cancer Treatment Reviews 2000; 26: 429-447

SUMMARY

As a consequence of the successful use of chemotherapy in the treatment of curable neoplasms such as germ cell tumors and malignant lymphomas, and the increasing application of neoadjuvant and adjuvant chemotherapy for various tumor types, the number of patients with a prolonged life expectancy after treatment is rising. Attention to long-term side effects, including cardiovascular toxicity is therefore of growing importance.

In this review, we evaluate the literature on long-term cardiovascular toxicity related to chemotherapy in adult patients. Two categories of patients with good life expectancy have been reviewed, namely patients treated with adjuvant chemotherapy. In the first category the literature on long-term cardiovascular morbidity in survivors of metastatic testicular cancer and lymphomas is discussed, while in the second category this is done for patients treated with adjuvant chemotherapy for breast and colon cancer. As well as the direct toxic effects of chemotherapy on the cardiovascular system, the indirect toxic effects such as chemotherapy related metabolic changes that may cause cardiovascular morbidity are also discussed.

INTRODUCTION

Various antineoplastic agents used in the treatment of cancer have adverse effects on the cardiovascular system. For frequently used agents, such as doxorubicin and 5-fluorouracil, the risk of acute cardiovascular toxicity (occurring during or shortly after its administration) has been extensively evaluated and is well-known.^{31,32} However, the long-term effects of many single agents and combination chemotherapy regimens on the cardiovascular system are not so well known. Results of various long-term follow-up series of survivors of childhood cancer treated with anthracycline-based chemotherapy regimens have made oncologists aware that cardiovascular damage can become manifest many years after completion of the chemotherapy.^{3,4,33} Also, for adult cancer patients late cardiovascular toxicity may be a threat. This seems, however, a less relevant issue for patients with disseminated cancer who have a poor prognosis, because most patients are likely to die before late cardiovascular toxicity becomes manifest.

For at least two categories of patients this late toxicity really may be a potential danger. The first category consists of patients who have been successfully treated with chemotherapy for metastatic disease and who are judged to have a normal life expectancy. In adult oncology this is confined to patients with metastatic testicular cancer and to a substantial portion of patients with malignant lymphomas.

The second category of patients for whom late cardiovascular toxicity might be a relevant issue are those who receive chemotherapy, not because of overt metastatic disease, but because of the risk of micrometastases, namely adjuvant treatment. Currently, the most important indication for adjuvant chemotherapy is breast cancer.³⁴ The other group in whom adjuvant chemotherapy plays an increasingly important role

is colon cancer.³⁴ Although adjuvant chemotherapy is used in other tumor types, this can not be considered standard treatment.

To evaluate the late effects of chemotherapy on the cardiovascular system we have studied the literature concerning the aforementioned groups. For each cancer type, cardiovascular toxicity will be described in relation to the most frequently used chemotherapy schedules. The main focus is late cardiovascular toxicity, but since the literature about acute cardiotoxic effects may give relevant information for the insights into the development of late cardiovascular toxicity, this will also be discussed briefly. In addition, factors indirectly influencing the development of cardiovascular disease, such as hypertension and metabolic changes that might have an impact on the development of late cardiovascular toxicity, will be discussed.

CARDIOVASCULAR TOXICITY IN SURVIVORS OF METASTATIC DISEASE

Survivors of metastatic testicular germ-cell cancer

Metastatic testicular cancer has become a curable disease after the introduction of cisplatin-containing chemotherapy in the late 1970s. At present more than 80 % of patients who present with metastatic testicular cancer can be cured.⁶ This high cure rate together with the fact that the tumor presents at a young age (usually between 20 and 40 years) makes germ cell cancer an important model for both curable disease and for long-term side effect of chemotherapy.

Chemotherapy regimens

Patients with metastatic testicular cancer were originally treated with cisplatin, vinblastine and bleomycin (PVB) followed by prolonged maintenance therapy, consisting of cisplatin and vinblastine.³⁵ Subsequent studies showed that this maintenance therapy was unnecessary,³⁶ and vinblastine was replaced by the less toxic and probably more efficacious agent etoposide.³⁷ At present, the regimen consisting of four cycles of bleomycin, etoposide and cisplatin (BEP) is accepted as the standard.³⁸

Acute cardiovascular toxicity

Myocardial toxicity

No clear indications exist that any agent (bleomycin, etoposide, cisplatin, vinblastine) used for testicular cancer, causes direct damage to the cardiomyocytes in a comparable way to anthracyclines. Myocardial infarctions that occur during chemotherapy usually have a vascular origin and are discussed below.

Vascular toxicity

Cardiovascular events

Several reports have appeared in the literature describing cases of severe angina pectoris and acute myocardial infarctions occurring during or shortly after administration

of chemotherapy for testicular cancer.^{10,12,39-41} In all these cases occurring in young men, there was no clear evidence of pre-existing cardiovascular disease and in those cases where a coronary angiography was performed, no abnormalities were found. Hypoperfusion of the coronary arteries resulting from vasospasm or thromboembolism seems the most probable mechanism underlying these acute ischaemic events. As magnesium plays an important role in the maintenance of the vascular smooth muscle tone, hypomagnesemia, which frequently accompanies cisplatin therapy,⁴² may provoke such vasospasms of the coronary arteries.²⁹ However, since myocardial infarctions have also been reported after the administration of both the single agent vinblastine and etoposide, this may not be the only mechanism involved.^{43,44}

Although thromboses and thromboembolic events during chemotherapy may be the result of tumor activity, cisplatin based chemotherapy also appears to provoke thrombus formation.⁴⁵⁻⁴⁹ Possibly cisplatin based chemotherapy causes direct damage to the endothelium resulting in a hypercoagulable condition. Progressive elevations in plasma levels of the von Willebrand factor (a marker of endothelial injury), have been described in 3 patients who developed vaso-occlusive complications during cisplatin based chemotherapy.⁵⁰ Altered endothelial integrity may initiate platelet adhesion and activation or cause disturbances in prostacyclin-thromboxane homeostasis, thereby promoting thrombotic vascular occlusion. Whether a single agent or the combination of agents is responsible for these acute cardiovascular events is not clear, since they have been reported following vinblastine alone,⁴³ etoposide alone,⁴⁴ vinblastine and cisplatin,⁵¹ PVB- combination^{10,40} and the BEP combination.¹²

Cerebrovascular events

Several cases have been reported of fatal and non-fatal cerebrovascular events that happened during or shortly after chemotherapy administration.^{10,12,40,52,53} The close temporal association between the administration of chemotherapy and these events suggests a causal relationship. In none of these cases an abnormal cerebral angiography was reported, and in most of these cases risk-factors for vascular disease were lacking. The suggested mechanisms that underlay these events are similar to those that may play a role in the occurrence of cardiovascular events, namely temporary vasospasms or thromboembolism.¹⁰

Other vascular events

Pneumonitis induced by bleomycin is a well-known side-effect of this agent and is characterized by diffuse alveolar damage leading to interstitial pneumonitis and fibrosis. Although the exact pathogenesis remains unclear and several mechanisms are involved, the initial site of pulmonary injury probably takes place in the endothelial cells lining veins and arteries, followed by lesions in the capillary endothelium.⁵⁴ Thereafter several changes occur, including the typical decrease in type I pneumocytes, migration of alveolar macrophages, followed by an exudative and finally a fibrotic response. The changes in several lung function parameters observed during bleomycin containing regimens are considered to reflect these changes. Although bleomycin is probably the most important agent that causes damage to the lung vasculature, lung function parameters also decrease in patients treated with EP suggesting that etoposide, and

more probably cisplatin also may cause damage to the lung vasculature.⁵⁵ In the majority of cases the decreases in lung function are completely reversible,⁵⁶ suggesting also reversibility of vascular damage.

Late cardiovascular toxicity

Myocardial toxicity

As mentioned earlier the chemotherapy used in the treatment of disseminated testicular cancer does not have a direct toxic effect on the myocardium. Although minor damage can not be excluded with certainty, so far no cases of cardiomyopathy or heart failure unrelated to ischemic heart disease have been reported. However, late myocardial infarctions may occur as a consequence of coronary artery disease as will be discussed below.

Vascular toxicity

Various manifestations of late vascular toxicity have been reported in survivors of testicular cancer. The mechanisms underlying these manifestations are not clear and may be multifactorial. Chronic endothelial damage is thought to play an important role in the development of late vascular toxicity.⁵⁷ Such endothelial damage may be caused particularly by bleomycin and possibly also by cisplatin. Various toxic effects of bleomycin on endothelial cells in vitro and capillaries and small arterioles in vivo have been described.^{54,58,59} Such endothelial damage may be a generalized process affecting different parts of the vascular system. Microalbuminuria, which is considered to reflect wide-spread endothelial dysfunction,^{60,61} was recently found to be present in 22% of long-term survivors of testicular cancer.⁶² In patients treated with bleomycin, capillary damage of the nailfold can be visualized by capillary microscopy, even in asymptomatic patients.⁶³ Damage of the autonomic nervous system that innervates the smooth muscle of the peripheral vessels, may also play a role particularly in the pathogenesis of Raynaud's phenomenon.⁵⁷ Similar damage may be caused by the agents cisplatin or vinblastine, both known to have neurotoxic side effects.⁷ Persistent hypomagnesemia due to cisplatin-induced renal magnesium wasting, could be another pathogenetic factor involved in the development of vascular toxicity.^{28,64-66} Finally, the chemotherapy-induced metabolic and hormonal changes may have an impact on late vascular toxicity particularly on the development of premature atherosclerosis.

Coronary vasculature

The most worrying type of late vascular toxicity is damage to the coronary arteries, which may be directly life-threatening as a consequence of myocardial infarction. The first report about chronic damage of the coronary arteries dates from 1979. It describes two young men treated for testicular cancer with PVB chemotherapy, who were found at autopsy to have severe coronary atherosclerosis, despite the lack of factors for atherosclerosis.⁶⁷ Several other cases of coronary artery disease and myocardial infarctions have been described occurring months to years after chemother-

apy.^{14,15,40,68,69} In some of these cases a causal relationship with the chemotherapy given appears strong, while in others such relation is difficult to establish because of the presence of confounding factors (tobacco-use, family history of coronary artery disease). Since coronary artery disease is an endemic, such events may be coincidental and unrelated to the chemotherapy. Therefore the discussion whether chemotherapy for testicular cancer leads to an increased risk of cardiac events is ongoing and not yet elucidated. Recently, we found in 87 long-term survivors that five patients had developed coronary artery disease 9-16 years after chemotherapy, at ages ranging from 30 to 42 years. Two patients developed a myocardial infarction, which was fatal in one, while three patients had angina pectoris. Compared with the background male population in the Netherlands the frequency of coronary artery disease in these long-term survivors was increased.⁶²

Digital vasculature

The most well-recognized occurrence of chronic small vessel damage in patients treated with chemotherapy for testicular cancer is Raynaud's phenomenon (RP). RP is a disorder characterized by transient episodes of vasoconstriction of the digital arteries, classically presenting with pallor due to ischemia, followed by cyanosis and then redness and pain caused by hyperemia as the circulation restores itself.⁷⁰ Since cold is a common provoking factor, particularly in cold climates, RP can be a severe clinical problem that influences the quality of life.⁷⁰

In 1977, RP was described in relation to chemotherapy for testicular cancer.⁷¹ In the first large report, Vogelzang et al. found that 37 % of the patients who had been treated either with vinblastine and bleomycin or with the PVB-combination developed RP.⁹ In these patients RP became clinically evident after a median follow-up of 10 months (range, 2-28 months). After a median follow-up of 18 months, approximately half of the affected patients noticed a gradual resolution of the symptoms, but the other half continued to experience RP each winter. In subsequent series with long-term follow-up RP has been reported to persist in 23 to 43%.^{14,15,66,69,72-75} In the long-term survivors of testicular cancer from our institution 35 % had developed RP, which was transient in 10 %, but remained persistent in 25% patients with constant severity 10 to 20 years after treatment.⁶²

The fact that RP has been reported in patients who had been treated with bleomycin alone,^{76,77} suggests a causative role of this drug. In addition, in almost all of the reported cases of RP following chemotherapy for testicular cancer bleomycin was administered. Cisplatin and vinblastine appear to have additive or maybe synergetic effects, as is indicated by the higher prevalence of RP found in patients treated with PVB compared to patients treated with VB only⁹ and the higher prevalence in patients treated with PVB compared to patients treated with BEP.¹⁴

Similar to the bleomycin-induced endothelial lesions that can be found in the lung vasculature, ranging from vacuolization to detachment and necrosis,⁵⁴ such lesions may also occur in the digital vasculature and give rise to the development of RP. The risk of RP seems especially high in smokers.^{9,40,78} Endothelium that already has been injured by tobacco use might be more vulnerable to additional damage induced by chemotherapy. In several studies, however, a significant correlation of tobacco-use

and the occurrence of RP is lacking.^{72,74,79}

Penile vasculature

Van Basten et al. reported that patients with RP after chemotherapy for testicular cancer more often had erectile dysfunction compared with those without RP.⁸⁰ Although erectile dysfunction in these patients can be caused by several mechanisms, the authors suggested that angiopathy of the penile arteries might be involved.

Renal vasculature / hypertension

Hypertension during long-term follow-up is present in 13-29% of the patients.^{14,15,62,69,73,74,81} Whether these figures are higher than expected has not been carefully evaluated so far. In the long-term survivors from our own institution a high percentage (39%) was hypertensive, defined as a systolic blood pressure above 150 mmHg and/ or a diastolic blood pressure above 95 mmHg and/or the use of anti-hypertensive medication.⁶² However, since hypertension is found in the general population and the incidence rises with age, not all cases of hypertension can be attributed to the chemotherapy with certainty. Furthermore, the mechanism underlying the development of hypertension in these patients is unclear.

In sporadic cases a renovascular cause was identified. A 30-year old man developed accelerated hypertension three months after completion of PVB therapy and a renal biopsy showed narrowing of the interlobular arteries and fibrin thrombosis in a majority of the afferent arterioles.⁸² In addition, in a 38-year-old man who developed hypertension after chemotherapy nonatherosclerotic partial occlusion of the major branches of the left renal artery was demonstrated.⁸³

As mentioned earlier, microalbuminuria was present in 22% of the long-term survivors that we have evaluated, reflecting endothelial damage at least present in the renal vasculature, but possibly also in other parts of the vascular system.^{60,62} The patients with microalbuminuria had higher blood pressures than the patients without microalbuminuria. However, for the majority of patients that develop hypertension after chemotherapy the cause is unclear and might be the consequence of several mechanisms. Increased plasma renin and aldosterone levels have been reported in normotensive survivors of testicular cancer, but whether these changes in the renin-aldosterone-angiotensin-axis contribute to the development of hypertension in the long-term is unclear.⁸⁴

Cerebral vasculature

The occurrence of late cerebrovascular events has been reported rarely. Doll reported a 22-year old man who developed a cerebrovascular accident 4 months after cessation of the chemotherapy.¹⁰ Several large long-term follow-up studies did not report any case of cerebrovascular events.^{13,14} In the cancer survivors that we have investigated, one patient developed a cerebrovascular event at the age of 41 years, 11 years after chemotherapy. He had hypertension and hypertriglyceridemia.⁶² At present it is not clear whether a relation exists between the chemotherapy and the occurrence of late cerebrovascular events. However, the high incidence of hypertension present in the long-term survivors may increase the risk of late cerebrovascular events.⁸⁵

Indirect toxicity

Several indirect side effects of the chemotherapy can have a chronic damaging effect on the cardiovascular system, and may contribute to the development of cardiovascular toxicity.

Metabolic changes

Hypercholesterolemia is a well-known risk factor for cardiovascular disease. Boyer et al. were the first group to notice that hypercholesterolemia was a common finding in testicular cancer survivors.⁷³ They found elevated (non-fasting) serum cholesterol levels (>5.2 mmol/L) in 20 out of 30 survivors of testicular cancer after a median follow-up of 75 months (range, 48-126 months). Subsequent prospective studies showed that post-treatment cholesterol levels rise in the majority of patients compared with pre-treatment levels.^{15,16} Such rise may partly be explained by low pre-treatment cholesterol levels, possibly due to tumor activity. Low cholesterol levels have been reported in relation with tumor activity in several types of cancer, including prostate, colon and lung cancer⁸⁶⁻⁸⁸ and may be due to an increase in the activity of LDL receptors.^{89,90} However, in cured testicular cancer patients, cholesterol levels 4 to 6 years after chemotherapy are higher compared to the general population and higher than testicular cancer patients treated with orchidectomy only.¹⁵

Berger et al. found 15 to 159 months after chemotherapy (non-fasting) cholesterol levels greater than 6.7 mmol/L in 32% of the patients. Age-stratified comparisons with reference groups showed significantly higher cholesterol levels especially in the 19-29 year old patients.⁹¹ We also found hypercholesterolemia (defined as fasting cholesterol level > 5.2 mmol/L or the use of lipid lowering medication) in 79% of the investigated survivors 10-20 years after chemotherapy.⁶²

The cause of the hypercholesterolemia in these patients is unknown. Alterations in gonadal function induced by chemotherapy might have an important impact on lipid levels.^{92,93} Elevated levels of the follicle stimulating hormone and the luteinising hormone are found in a large percentage of the patients. Particularly the luteinising hormone levels rise as a consequence of a partial Leydig cell insufficiency, to maintain the testosterone levels at a physiological level (compensated state) but this mechanism fails in some patients (decompensated state) resulting in hypogonadism.⁹⁴ Hypogonadism is known to be associated with elevated serum levels of total cholesterol, LDL cholesterol, and triglycerides, although the exact mechanism is unknown.⁹⁵

Obesity is another factor that may influence the development of cardiovascular disease. It is an independent cardiovascular risk-factor as recently once again was demonstrated.⁹⁶ Gieterma et al. reported that the mean body mass index for all patients 4-6 years after chemotherapy was 2.8% higher than expected but returned to normal 7 to 10 years after chemotherapy.¹⁵

We recently found in 21% of the long-term survivors insulin resistance as measured by an increased insulin-to-glucose ratio. In some of these patients this insulin resistance might be related to lowered testosterone levels as a consequence of gonadal toxicity.⁹⁷ Furthermore, 22% of these patients had an increased urinary albumin excretion (microalbuminuria),⁶² which is considered as an utterance of endothelial

dysfunction and an independent risk-factor for cardiovascular morbidity.^{61,98} The combination of obesity, microalbuminuria, hypertension, insulin resistance and dyslipidemia suggests the presence of a metabolic syndrome-X-like state⁹⁹ in some of these patients. The development of these unfavorable risk-factors and metabolic alterations may have an important indirect impact on the development of cardiovascular disease in the long-term.

Survivors of metastatic lymphomas

Hodgkin's disease (HD)

After the introduction of successful chemotherapy and radiotherapy regimens, the treatment of HD has changed dramatically. Radiotherapy is curative for localized HD and advanced HD can be cured by chemotherapy. Depending on the stage of the disease, patients may be treated with radiotherapy alone, with chemotherapy alone or with a combination of them. Currently, long-term disease free survival rates range from 75% in advanced stages to 90 % in early stages of the disease. The tumor can occur at any age, however a peak incidence exists between 20 and 40 years and above 60 years of age.¹⁰⁰ In young survivors, the possible long-term consequences of treatment are of particular concern.¹⁰¹

Chemotherapy regimens

Since its introduction in 1970, the MOPP-regimen (mechlorethamine, vincristine, procarbazine, and prednisone) has been the standard chemotherapy for Hodgkin's disease.¹⁰² The development of second malignancies as a late complication of this chemotherapy combination has restricted its use in recent years. For that reason other regimens were developed notably ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) schedule.¹⁰³ Other combinations have used doxorubicin analogues such as epirubicin or mitoxantrone, mainly to reduce the risk of cardiotoxicity. Combination regimens such as the alternating MOPP/ABVD and the MOPP/ABV hybrid regimen are also current standard programs.

Non-Hodgkin's lymphoma (NHL)

The development of curative combination chemotherapy for patients with advanced stage of NHL has been another major success of cancer therapy in recent decades.

Chemotherapy regimens

For patients with advanced intermediate-grade or high-grade NHL the cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP)-regimen is considered the standard therapy.¹⁰⁴ With chemotherapy only, most patients with localized disease (stage I or II) can be cured, and 30-40% of patients with advanced disease (stage III or IV).¹⁰⁵ Recognizing the need to improve these results, new and more complex regimens were developed in the 1980s such as the methotrexate, leucovorin, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone (M-BACOD)-combination and the methotrexate, leucovorin, doxorubicin, cyclophosphamide, vincris-

tine, prednisone, and bleomycin (MACOP-B)-combination. However, after the landmark phase III trial published by Fisher et al. in which several regimens were compared, most oncologists use CHOP as their gold standard for newly diagnosed patients with aggressive NHL.¹⁰⁴ The success of the chemotherapy in the treatment of lymphomas has resulted in a growing number of long-term survivors.

The study of long-term consequences of the chemotherapy in NHL is more complicated compared to survivors of HD, because the peak incidence of NHL is above 40 years of age and for several subtypes even above 60 years of age.¹⁰⁶ As the incidence of cardiovascular morbidity increases above 50 years of age, it is more difficult to establish whether cardiovascular morbidity occurring during the follow-up, is the result of chemotherapy or the consequence of aging. Compared to the multitude of studies on long-term consequences of treatment in survivors of HD, the literature about long-term consequences of therapy in NHL is limited.

Acute cardiovascular toxicity

Myocardial toxicity

Anthracyclines are frequently used nowadays as part of the cytostatic treatment of both HD and NHL. Although the initial anthracycline-induced damage to the myocytes probably occurs shortly after its administration, overt heart failure presents usually weeks, months or years after chemotherapy. Therefore this phenomenon will be discussed further in the section on late cardiovascular toxicity. Acute cardiotoxic effects that can occur during or shortly (within hours) after administration of anthracyclines include ECG changes (such as non-specific ST and T-wave changes, decreased QRS voltage, and prolongation of the QT interval) and rhythm disturbances (such as sinus tachycardia, and supraventricular arrhythmias).¹⁰⁷ These changes appear of minor clinical relevance as they rarely happen with current treatment regimens and do not predict chronic cardiotoxicity.¹

The other agent that can cause acute cardiotoxicity is cyclophosphamide, which is mainly cardiotoxic at high doses, such as those used before bone marrow or stem cell transplantation. The reported cardiotoxicity ranges from transient ECG changes and asymptomatic increases of serum levels of cardiac enzymes to more severe cardiotoxicity such as exudative pericardial effusion, ventricular hypertrophy and fatal myopericarditis and (hemorrhagic) myocardial necrosis.¹⁰⁸⁻¹¹⁰ The onset of the latter types of cardiotoxicity is acute with death within 15 days. The cardiotoxic effects of cyclophosphamide can probably partly be explained by the damage to the endothelium of the myocardial capillaries with resultant increase in permeability and extravasation of plasma, red blood cells and toxic metabolites.¹¹¹ This in turn can cause myocyte damage and interstitial hemorrhage and edema. The cardiotoxicity of cyclophosphamide may be potentiated by anthracycline administration.^{112,113} Various cases of high-dose cyclophosphamide associated acute cardiotoxicity in NHL and HD patients have been reported,^{114,115} but the exact incidence of this problem is not known.

*Vascular toxicity**Cardiovascular events*

Although rarely, a number of patients have been described who developed acute myocardial infarctions during or shortly after chemotherapy for both HD and NHL.^{116,117} In these reports the cause of these events is attributed to the administration of vinca alkaloids (vincristine or vinblastine). The mechanism by which chemotherapy, specifically vinca alkaloids cause myocardial infarctions is speculative. Suggested mechanisms include the inducement of a hypercoagulable diathesis, and the occurrence of coronary artery spasms. Possibly vinca alkaloids or one of the other chemotherapeutic compounds given produce cellular hypoxia predisposing the vessel to spasm. Alternatively, a combination of neural, vascular, and thrombotic events may be involved.¹¹⁷

Other vascular events

There are at least two other manifestations of acute vascular toxicity associated with chemotherapy for lymphomas. As discussed earlier, bleomycin-containing regimens bear the risk of inducing bleomycin pneumonitis, which is thought to be a result of damage to the endothelium in the lung vasculature. Hirsch et al. reported a decline of the pulmonary function tests in 22 of 60 (37%) patients with HD after completion of six cycles of ABVD. Particularly the forced vital capacity and the diffusing capacity of carbon monoxide were significantly reduced compared to the pre-treatment values.¹¹⁸

Another manifestation of acute vascular toxicity may be the thromboembolic events that occur during chemotherapy. Of the 177 HD patients evaluated by Seifter et al., seven experienced thrombotic events in lower (2) or upper extremities (5) during chemotherapy (MOPP or MOPP/ABVD).¹¹⁹ Particularly the uncommon high proportion of upper extremity thrombosis suggests chemotherapy-induced acute vascular toxicity that results in a hypercoagulable state, although tumor activity as the cause of these events can not be excluded. Other indications for an association of chemotherapy with thromboembolic events were reported by Clarke et al. who evaluated 85 NHL patients. While thromboembolic events occurred in 4 of the 11 NHL patients who had received weekly chemotherapy, this was not the case in patients treated with less intensive schedules.¹²⁰

*Late cardiovascular toxicity**Myocardial toxicity*

Of the agents used in the treatment for HD and NHL, anthracyclines are the most important ones in causing direct injury to the myocardium. The exact mechanism how anthracyclines affect the myocardium is not fully elucidated, but the formation of free radicals, and calcium overload in the myocytes play an important role.¹⁰⁶ These processes lead to patchy myocyte damage, starting with myofibrillar loss, swelling and disruption of mitochondria and the sarcoplasmic reticulum leading to intramyocyte vacuolization and eventually myocyte death. As a consequence, cardiomyopathy de-

velops which can result in congestive heart failure (CHF).¹ However, since the heart has considerable compensatory reserves, a long time may elapse before CHF becomes manifest, if at all.

Anthracycline-induced CHF can occur weeks, months or many years after administration, but the majority of cases presents within one year.² The risk of inducing late anthracycline cardiotoxicity strongly increases with the cumulative dosage given, but a wide variation exists in interindividual susceptibility for anthracycline cardiotoxicity. In the landmark study by Von Hoff total doses of doxorubicin of 400 mg/m² body surface area were associated with an incidence of CHF less than 2%, while this incidence increases to 7% at a dose of 550 mg/m², and to 18% at a dose of 700 mg/m².² However, since the mean time of observation was limited (581 days) these incidence rates might not reflect the long-term risk.

In long-term follow-up studies in survivors of childhood cancer, incidences of subclinical cardiotoxicity in 23% up to approximately 60% have been reported, increasing with the length of follow-up duration.^{3,33} In addition, cases of CHF presenting many years after treatment have been reported in both children and adults.^{4,121} Since a young age (particularly younger than 4 years) is an additional risk-factor for the development of anthracycline cardiotoxicity,¹²² these figures can not precisely be extrapolated to adults, but they illustrate that the risk of CHF continues long after treatment.

Although high-dose cyclophosphamide can cause acute cardiotoxicity, late cardiotoxicity in the first year after high-dose chemotherapy and bone marrow-transplantation seems exceptional, as was recently described by Ghielmini et al.¹²³ However, longer follow-up is needed to exclude long-term cardiotoxicity with certainty.

Hodgkin's Disease

In contrast to the abundant data detailing the long-term risks of cardiac damage induced by radiotherapy, there are only a few studies that focus on the long-term risk of chemotherapy-induced cardiotoxicity. Since a large proportion of patients is treated with mediastinal irradiation plus chemotherapy, the exact risk of anthracycline-cardiotoxicity is difficult to determine. The successful development of the ABVD regimen has raised concerns about the occurrence of anthracycline-induced cardiac injury in long-term survivors. However, in most treatment protocols the cumulative dose of doxorubicin is 300 mg/m² or less, which carries a low probability of CHF.

Santoro et al. evaluated the seven-year results of 232 HD patients treated with radiotherapy and either MOPP or ABVD chemotherapy. Only one patient died of congestive heart failure that might have been related to the administration of doxorubicin (293 mg/m²), although radiotherapy was more likely the main cause. In 50 young patients (mean age 32 years) in complete remission for more than 5 years, evaluation by both echocardiography and MUGA-scans revealed a similar mean left ventricular ejection fraction (LVEF) in the ABVD and MOPP group, but the mean end-diastolic left ventricular diameter was higher in the ABVD group.¹²⁴ Also in a large series in which 415 patients were treated with two different MOPP/ABVD regimens followed by radiotherapy, cardiac failure was not reported as a cause of death. Three patients died of a myocardial infarction, but there was no definitive evidence that this was related to the administered treatment.¹²⁵ So a cumulative dose of doxorubicin of less than 300

mg/m² does not appear to induce a high risk of developing CHF, however, the possibility of mild anthracycline cardiotoxicity has not been evaluated adequately.

To determine the precise effect of chemotherapy on the cardiac function, prospective monitoring is needed. Few studies have been published so far. In a small study in 24 patients with a median follow-up of 6.3 years after MOPP/ABVD or ABVD (mean cumulative dose doxorubicin 233 mg/m²) combined with radiotherapy Sal-loum et al. found only one patient with an abnormal LVEF. Although for the whole group of patients a significant decrease was found of 4.7% absolute EF units compared with pre-treatment values, this did not result in cardiac symptoms.¹²⁶ Aviles et al. analyzed the cardiac function in 136 patients 5 to 8 years after either ABVD (median dose doxorubicin 475 mg/m²) or epirubicin-BVD (median dose epirubicin 410 mg/m²) or mitoxantrone-BVD (median dose mitoxantrone 125 mg/m²). Cardiac toxicity, defined as an LVEF less than 45% or a decrease of LVEF by more than 15 % of the pre-treatment levels, was found in 51 patients (37%). Ten of these patients developed non-fatal cardiac failure.¹²⁷ As expected, higher doses of anthracyclines resulted in a higher risk of cardiomyopathy.

Non-Hodgkin's Lymphoma

In the CHOP regimen the cumulative dose of doxorubicin is 400- 500 mg/m² and therefore the expected incidence of anthracycline induced CHF will be higher compared to the regimens given for HD. In a recent study by Miller et al. left ventricular dysfunction (defined as clinical symptoms of CHF or as a decrease of more than 20% in the LVEF) was found in 7 of 201 patients treated with 8 cycles of CHOP (cumulative dose doxorubicin 400 mg/m²) while in the group treated with 3 cycles combined with radiotherapy none of 200 patients developed left ventricular dysfunction.¹⁰⁵ Seven patients treated with 8 cycles of CHOP but only two treated with 3 cycles of CHOP plus radiotherapy subsequently died of apparent heart disease.

Haddy et al. studied 103 young survivors treated with four different regimens (doxorubicin containing and non-containing) with a follow-up up to 20 years. In eight of 57 patients (doxorubicin doses greater than 200 mg/m²) echocardiographically determined left ventricular dysfunction was identified. Four were symptomatic of whom two needed cardiac medication, while the other four were asymptomatic.¹²⁸ In older patients late cardiotoxicity can also occur as was described by Freter et al.¹²⁹ in a report on a 60-year old woman who developed CHF seven years after CHOP with a cumulative dose of doxorubicin of 450 mg/m². Cardiac biopsy specimens showed abnormalities characteristic for anthracycline-cardiotoxicity. However, the incidence of anthracycline-induced CHF in older patients with NHL is probably underreported in the literature, because cardiac events occurring at an old age are much less likely to be reported and ascribed to chemotherapy compared to such events occurring in young patients.

Irradiation of the heart may contribute to the risk of anthracycline-induced CHF.^{3,130} So far, follow-up studies of cardiac function in HD patients who received combined modality treatment including anthracycline-containing chemotherapy have not shown an increased risk of clinical cardiomyopathy.^{124,131,132} However, further follow-up is necessary to determine this risk in the long-term.

Vascular toxicity

Reports on late vascular toxicity associated with chemotherapy alone for lymphomas are limited. In contrast to the use of bleomycin in chemotherapy for testicular cancer, the use of bleomycin in the ABVD regimen does not appear to result in utterances of vascular toxicity, such as Raynaud's phenomenon. This might be due to the lower total dose of bleomycin used in the ABVD regimen (120 mg/m²) compared to the total dose used in chemotherapy regimens for testicular cancer (approximately 180 mg/m²) or to the absence of toxicity amplifying drugs. As has been discussed in the section late vascular toxicity in germ cell cancer, bleomycin is considered to be the causative agent, although vinca-alkaloids or cisplatin can amplify this toxicity.

Coronary vasculature

In long-term survivors of HD, damage to the coronary arteries seems to be caused mainly by radiotherapy. Mediastinal irradiation is known to affect different components of the heart including coronary arteries.¹³³ In a large series with 2232 HD patients with a mean follow-up of 9.5 years after treatment, Hancock et al. reported that mediastinal radiotherapy resulted in a 3.2 fold increased risk of death from myocardial infarction compared to the general population, probably due to the development of premature coronary artery atherosclerosis.¹³⁴ This and other large follow-up series^{135,136} were unable to find an association between chemotherapy and death from myocardial infarction, suggesting that chemotherapy alone does not affect the coronary arteries directly. Since cardiac deaths were end points in these trials, risk of coronary artery disease might be under estimated and therefore not detected.

Pulmonary vasculature

A manifestation of vascular toxicity reported in association with HD and its treatment is pulmonary veno-occlusive disease (PVOD). PVOD is a rare cause of pulmonary hypertension of unknown aetiology.¹³⁷ The diagnosis is usually made by histological examination of lung tissue obtained either at open lung biopsy or at necropsy. Such examination characteristically reveals occlusion of pulmonary veins and venules by loose fibrous material or collagen-rich connective tissue. Several cases of PVOD occurring after chemotherapy have been described, including cases of lymphomas treated with MOPP and COPP respectively.¹³⁸ In the latter case there was a 10-year interval between the administration of chemotherapy and the onset of PVOD. PVOD has also been reported in two patients with lymphocytic lymphomas treated with bleomycin and prednisone.¹³⁹ Although the number of cases in the literature is limited, this may be due to the failure to recognize this complication.¹³⁹

Indirect toxicity

Gonadal dysfunction is a well-known complication of cytostatic treatment of HD, particularly after regimens containing alkylating agents. The destruction of germ cells in women can result in loss of steroid producing cells, which in turn causes reduction of circulating serum estrogen levels and an increase in serum LH and FSH levels.¹⁰¹ Fe-

male patients over the age of 40 at the time of chemotherapy frequently will have a premature menopause, while most patients under the age of 30 will remain fertile and eventually have return of normal menses. The incidence of coronary artery disease in the general population is low in premenopausal women, and rises in postmenopausal women.¹⁴⁰ This is probably due to a decrease in circulating estrogen levels that have a protective effect on the cardiovascular system and to the occurrence of an unfavorable lipid profile.¹⁴¹ These latter changes are considered to be atherogenic and to contribute to the increased risk of coronary artery disease. The adverse effects of menopause on serum lipid levels are reversed by estrogen hormone replacement therapy, which is also associated with the reduction of coronary heart disease.¹⁴² Bilateral oophorectomy in premenopausal women has been shown to result into unfavorable changes in lipid levels such as a rise in lipoprotein (a), apoprotein-B, LDL-cholesterol and total cholesterol and a decrease in HDL-cholesterol.¹⁴³⁻¹⁴⁵ Similar to surgical castration, chemical castration of the ovaries can result in comparable unfavorable changes in the lipid profile. Therefore, it is plausible that chemotherapy induced premature menopause also increases the risk of premature atherosclerosis.

In contrast to other regimens the MOPP regimen induces azoospermia in the majority of male patients. This azoospermia is also accompanied by elevations of serum LH and particularly FSH levels, indicating Leydig cell damage. These consequences of gonadal dysfunction in males may also have a detrimental effect on the serum lipid levels. However, lipid levels in long-term survivors of lymphomas have hardly been studied. Munker et al. found that serum total cholesterol and triglycerides levels of 155 patients with Hodgkin's disease in complete remission for a mean duration of 5.7 years, did not differ from the general German population.¹⁴⁶ To make sure that chemotherapy in survivors of lymphomas indeed does not effect the lipid levels, these findings should be confirmed in other studies with control groups. Until then an unfavorable lipid profile resulting from direct gonadal toxicity should be considered as a potential hazard for cardiac disease after chemotherapy in young lymphoma survivors.

CARDIOVASCULAR TOXICITY AFTER ADJUVANT CHEMOTHERAPY

Breast cancer

Apart from surgery and radiotherapy, adjuvant chemotherapy nowadays plays an essential role in the treatment of high risk breast cancer.³⁴ Whereas single-agent adjuvant chemotherapy generally has a marginal effect on survival, combination chemotherapy has shown to be more successful. One of the most widely used combinations is the 'cyclophosphamide, methotrexate, 5-fluorouracil' (CMF)-combination which was introduced in the 1970s.¹⁴⁷ The use of anthracyclines in adjuvant combination chemotherapy has been shown further to reduce the recurrence rate and to improve the survival.¹⁷ Anthracycline containing combinations currently used or evaluated include cyclophosphamide, doxorubicin, 5-fluorouracil (CAF),¹⁴⁸ doxorubicin, cyclophosphamide (AC)¹⁴⁹ and 5-fluorouracil, epirubicin and cyclophosphamide (FEC).^{18,150}

*Acute cardiovascular toxicity**Myocardial toxicity*

Anthracyclines can cause direct damage to the myocardium, but the clinical manifestation of this myocardial damage usually becomes manifest after cessation of the chemotherapy and will be further discussed in the next section. The clinically less relevant acute effects of anthracyclines have been discussed earlier. 5-Fluorouracil also appears to have direct toxic effects on the myocardium, although toxic effects on the coronary arteries probably are the main pathogenetic factor. Necropsy findings in humans who died from fatal myocardial infarction or cardiogenic shock after 5-fluorouracil therapy, showed patchy myocardial necrosis and mononuclear inflammation unrelated to the distribution of the coronary arteries.¹⁵¹ A direct interference of 5-fluorouracil with the myocardial cell metabolism leading to cellular hypoxia has been suggested from a study with an isolated perfused rat heart model.¹⁵² In addition, cases of dilative cardiomyopathy, and congestive heart failure have been reported after 5-fluorouracil treatment, suggesting also a direct toxic effect on the myocardium.¹⁵³⁻¹⁵⁷

Cyclophosphamide, used in most adjuvant regimens for breast cancer is mainly cardiotoxic at high doses, such as used before bone marrow or stem cell transplantation as was discussed in the section on lymphomas. Several cases of cyclophosphamide related fatal cardiotoxicity have been described during high-dose chemotherapy for breast cancer.^{108,110} There are no indications that the agent methotrexate has toxic effects on the myocardium.

*Vascular toxicity**Cardiovascular events*

Of the agents used in adjuvant chemotherapy combinations for breast cancer, probably only 5-fluorouracil has toxic effects on the coronary arteries. The most common clinical manifestation of 5-fluorouracil associated cardiovascular toxicity is angina-like chest pain, but cases of myocardial infarction and cardiogenic shock have also been reported.^{32,158} Most incidents occur during or within several hours up to days after starting 5-fluorouracil treatment. The main pathogenetic factor for these acute cardiovascular events is probably the occurrence of coronary vasospasm, which assumption is based on the characteristic clinical and electrocardiographic presentation of reversible ischemia without intraluminal obstruction of the coronary arteries.¹⁵⁹ However, endothelial cell damage and consequent thrombus formation may play a role.¹⁶⁰ Cardiovascular toxicity seems more common in patients treated with high-dose continuous infusion.¹⁵⁹ A clear relation with the cumulative dose 5-fluorouracil administered has not been identified.¹⁵⁷ Previous or current radiation involving the heart has also been proposed as a risk factor for the development of 5-fluorouracil-associated cardiotoxicity, particularly as 5-fluorouracil possesses radiosensitising properties.¹⁶¹ Although cases of 5-fluorouracil related cardiovascular toxicity also have been reported during adjuvant chemotherapy for breast cancer,¹⁶² the precise incidence is uncertain.

The cardiotoxic effects of cyclophosphamide probably result from direct effects on the myocardial capillaries. Effects on the coronary arteries do not seem to be involved, although such effects can not be fully excluded. There are no indications that the agent methotrexate has adverse effects on the cardiovascular system.

Other vascular events

As was mentioned earlier in the sections on testicular cancer and lymphomas, thromboembolic events during chemotherapy may be the result of acute vascular toxicity. Levine et al. reported an incidence of thromboembolic events of 6.8% during adjuvant chemotherapy for stage II breast cancer, while no events occurred after completion of chemotherapy (median follow-up duration 20 months).¹⁶³ Comparable results were reported by Weiss et al. who found an incidence rate of thromboembolic events of 4.2 to 7.2% in stage II breast cancer patients treated with different adjuvant chemotherapy schedules.¹⁶⁴

Thromboembolic events occurring during adjuvant chemotherapy are not likely to be caused by tumor activity, and therefore a causal relation with the chemotherapy appears strong. Direct damage to endothelial cells resulting in a hypercoagulable state may cause these events.¹⁶⁵

Late cardiovascular toxicity

Myocardial toxicity

As discussed earlier anthracyclines are well-known for their myocardial damage, resulting in cardiomyopathy with left ventricular dysfunction, which can lead to congestive heart failure (CHF). The most important factor determining the risk of late CHF is the cumulative dose of the anthracyclines given. In most adjuvant regimens this cumulative dose is kept rather low, below the 'safe threshold value' of 450-550 mg/m² for doxorubicin and 900-1000 mg/m² for epirubicin.

Buzdar et al. reported that, after a median follow-up duration of 68 months the incidence of doxorubicin-associated CHF was 1% (five out of 534 patients) in the FAC regimen when a cumulative dose of 300 mg/m² was used.¹⁶⁶ The incidence increased to 4% (five out of 134 patients) when a cumulative dose of 450 mg/m² was administered. The longest interval from the end of the doxorubicin therapy to the development of CHF was 33 months. In a group of 1492 breast cancer patients treated with the AC combination (cumulative dose doxorubicin 240 mg/m²) only 0.2% asymptomatic and 0.1% symptomatic cardiac dysfunction was reported and no cardiac death after a follow-up duration of 3 years.¹⁴⁹ The elevated risk of cardiac failure with increasing cumulative dose of doxorubicin was also demonstrated in a recent trial by Shapiro et al. in which breast cancer patients received either five or 10 cycles AC, resulting in a cumulative dose doxorubicin of 225 and 450 mg/m² respectively. In contrast to the AC5 regimen, the AC10 regimen showed an 3.6 fold increased risk of cardiac events (CHF and myocardial infarctions) compared with the general population (Framingham Heart Study data).¹⁶⁷ Adjuvant regimens that contain the less cardiotoxic

anthracycline analogue epirubicin result in low incidence rates of CHF. Coombes et al. reported in a randomized trial of FEC versus CMF in premenopausal women, only one case of non-fatal CHF out of 380 patients treated with the FEC regimen (cumulative dose epirubicin 300-400 mg/m²) after a median follow-up duration of 4.5 years.¹⁵⁰ In another randomized trial with higher cumulative doses of epirubicin (mean 608 mg/m²) no case of CHF was reported in 351 patients treated with FEC after a median follow-up of 59 months.¹⁸

In the above mentioned studies the description of cardiotoxicity is mainly based on patient records so these studies could underestimate the incidence of mild cardiac dysfunction which can only be established by careful cardiac monitoring and longer follow-up. An interesting study in this respect was recently presented by Gianni et al. concerning a randomized trial of adjuvant CMF with or without doxorubicin (75 mg/m² per cycle) in 1000 breast cancer patients.¹⁶⁸ A subset of 329 patients who were continuously disease free for a median of 10.8 (5-14.6) years, underwent cardiac evaluation including echocardiography. Of these patients 150 had received CMF alone and 179 CMF plus doxorubicin (median cumulative dose of 294 mg/m²). While the incidence of CHF for both treatment groups did not differ, the incidence of left ventricular dysfunction, defined as pathological or borderline LVEF, was 7.9% in the anthracycline treated patients vs. 0.7% in the patients treated with CMF alone.¹⁶⁸ Whether patients with subclinical left ventricular dysfunction eventually develop CHF is uncertain and can only be established by further follow-up.

Although both 5-fluorouracil and cyclophosphamide might have chronic adverse effects on the myocardium in individual patients, this appears to be rare with the adjuvant regimens used for breast cancer.

Vascular toxicity

To our knowledge none of the agents used in adjuvant combination chemotherapy for breast cancer appears to cause significant chronic damage to any part of the vascular system. Also, the acute toxic effects of 5-fluorouracil on the coronary arteries do not seem to result in chronic coronary artery disease.³²

Adjuvant radiotherapy

Whether irradiation of the mediastinum and / or chestwall further increases the risk of late cardiotoxicity is a point of discussion complicated by the changes in cumulative radiation dosages and in radiation fields, resulting from modern irradiation techniques. Initially, a meta-analysis showed an excess of late deaths among women who received postoperative radiotherapy compared with those treated with surgery alone.¹⁶⁹ However, a subsequent meta-analysis did not detect a difference in overall mortality,¹⁷⁰ although analysis of cause-specific mortality showed an excess of late cardiac death among irradiated patients. A causal relationship between irradiation and death from myocardial infarction is also suggested when the differences in cardiac mortality after left-sided breast cancer and after right-sided breast cancer are taken into account.¹⁷¹ Whether the difference is related to damaging the myocardium directly or indirectly

by damaging the left anterior coronary artery has not yet been elucidated.¹⁷² Using modern breast irradiation, small daily fractions and CT-based field arrangements, no increased long-term risk of cardiac mortality was found.¹⁷³

Moreover, recently Højris et al. could not detect a difference in morbidity nor mortality from ischemic heart disease between surgery and surgery plus radiotherapy over a median follow-up of 10 years (0.9% vs. 0.8%).¹⁷⁴ Nowadays, most premenopausal and menopausal patients are treated with a combination of chemotherapy and radiotherapy. Evaluating the interaction between both adjuvant treatments, Højris et al. did not find a difference in the rate of ischemic heart disease whether or not radiotherapy was combined with CMF or with tamoxifen.¹⁷⁴

Late cardiotoxicity of the combination of radiotherapy with anthracycline based chemotherapy seems to be more pronounced. Valagussa et al. reported after a median follow-up duration of 7 years an overall incidence of 0.8% CHF among 501 breast cancer patients treated with cumulative doses of doxorubicin of 300 mg/m².¹⁷⁵ In a subset of 116 patients with left-sided breast cancers treated with concurrent breast radiation, the incidence of CHF increased to 2.6%, while CHF was not observed in patients with right-sided breast cancers nor in those treated with non-anthracycline-containing chemotherapy regimens. In addition, Shapiro et al. found that the increased risk of cardiotoxicity associated with higher cumulative doses of doxorubicin applied especially to patients who were treated with higher dose-volumes of radiotherapy to the left-side of the chest-wall.¹⁶⁷ However, since others have not detected increased cardiotoxicity in breast cancer patients treated with radiotherapy and doxorubicin compared to those treated with doxorubicin alone,¹⁷⁶ this possible amplifying effect of radiotherapy on the risk of anthracycline cardiotoxicity needs further study.

Indirect toxicity.

As for survivors of testicular cancer and lymphomas, gonadal dysfunction is a frequent side effect of adjuvant chemotherapy given to premenopausal breast cancer patients. In adjuvant regimens for breast cancer, the alkylating agent cyclophosphamide is most commonly associated with ovarian damage. Based on a recent literature review, Bines et al. found that the average incidence of chemotherapy related amenorrhea for CMF-based regimens is 40% for women younger than 40 years of age and increases to 76% for those over 40 years of age.¹⁷⁷ The incidence for other adjuvant regimens is not well established, but according to the experience of the authors of this review, the AC regimen results in a lower rate of amenorrhoea (34%). As was discussed in previous sections, chemotherapy-induced premature menopause may increase the risk of cardiovascular disease partly due to the occurrence of unfavorable changes in the lipid profile.

Saarto et al. studied the effect of ovarian failure one year after CMF chemotherapy on the lipid profile in 57 patients who were premenopausal before treatment.¹⁷⁸ In 24 (42%) patients who subsequently developed amenorrhea, the serum total cholesterol, the LDL cholesterol and the apolipoprotein B level increased by 15%, 18% and 12 %, respectively. However, a favorable effect on the HDL cholesterol level was observed, namely an increase by 15%. Similar but less pronounced changes

in the lipid profile were observed in the patients who developed irregular menstruation, whereas the patients who maintained regular menstruation without hormonal changes did not show these changes in lipid profile.

The effect of these changes might in the long-term increase the risk of cardiovascular disease.

The unfavorable changes in the lipid profile seems to be reversed in patients who receive the estrogen antagonist tamoxifen. Following adjuvant chemotherapy treatment, nowadays many breast cancer patients are additionally treated, for periods of 2-5 years. Tamoxifen has effects on serum lipoprotein levels which resemble those of estrogens. Tamoxifen persistently lowers serum levels of total cholesterol, LDL cholesterol, and lipoprotein (a).^{179,180} Such tamoxifen-induced favorable changes in the lipid profile may explain the observed reduction in cardiovascular morbidity associated with tamoxifen use.^{181,182} On the other hand, some studies have reported an increase in serum triglycerides by 20-30%,^{183,184} which in rare cases can lead to severe hypertriglyceridemia with fatal consequences including pancreatitis and multiorgan failure.^{185,187} However, the net effect of tamoxifen on lipid levels is advantageous in the majority of breast cancer patients, thereby probably lowering the risk of cardiovascular morbidity.

Colorectal cancer

Over the last two decades the use of adjuvant 5-fluorouracil based chemotherapy in the treatment of high-risk colorectal cancer has been shown to improve local control and survival.^{188,189} 5-Fluorouracil is given alone or in combination with various agents including folinic acid or levamisole.

Acute cardiovascular toxicity

Myocardial toxicity

Of the agents used in the adjuvant chemotherapy for colorectal cancer only 5-fluorouracil is known to have toxic effects on the cardiovascular system (v.s. under breast cancer). The literature about cardiotoxicity in colorectal cancer patients treated with 5-fluorouracil based chemotherapy in the adjuvant setting is limited. In a small prospective study, Grandi et al. evaluated the cardiac function of 16 patients who were treated with 6 cycles of 5-fluorouracil 400 mg/m², and folinic acid 20 mg/m². Systolic parameters such as the peak lengthening rate of the left ventricular diameter and the peak thinning rate of left ventricular posterior wall, were decreased at the end of treatment, but 6 months after treatment they returned to normal values and were similar to pre-treatment levels. It is not clear how these results should be interpreted, but they possibly reflect slight, reversible myocardial toxicity.¹⁹⁰ In addition, a case of reversible cardiac dysfunction with pulmonary congestion during 5-fluorouracil chemotherapy has been reported, also suggesting acute myocardial toxicity.¹⁵⁵

*Vascular toxicity**Cardiovascular events*

Schober et al. studied the occurrence of 5-fluorouracil related cardiovascular toxicity in 390 patients treated for colorectal cancer.¹⁵⁵ Cardiovascular toxicity presented in eight patients as angina pectoris and one patient who developed a fatal myocardial infarction. All but one patient had a history of cardiac or vascular morbidity, suggesting an increased risk for such patients.

Other vascular events

A rare case of Raynaud's phenomenon after 5-fluorouracil treatment for rectal cancer has been described in a 58 year old man.¹⁹¹ After 7 cycles he developed severe Raynaud's phenomenon with digital ischemia and the development of a digital infarct. Nailfold microscopy revealed abnormal capillaries. This indicates that 5-fluorouracil besides effects on the cardiac vasculature, can cause also toxicity in other parts of the vascular system.

Late cardiovascular toxicity

The clinical prognosis of 5-fluorouracil cardiotoxicity is usually good, with most clinical symptoms, electrocardiographic or echocardiographic alterations being fully reversible. The mean resolution time of symptoms ranges from minutes up to 2 days. Once recovered, there appear to be no late sequelae after most incidents.³² However, this needs further follow-up, especially in those patients that have experienced severe cardiovascular toxicity.

Indirect toxicity

5-fluorouracil-based chemotherapy for colorectal cancer seems not to result in significant indirect toxicity that contributes to long-term cardiovascular morbidity.

DISCUSSION

As a consequence of the successful use of chemotherapy in the treatment of curable neoplasms like germ cell tumors and malignant lymphomas, and the increasing application of neoadjuvant and adjuvant chemotherapy for various tumor types, the number of patients with a normal life expectancy after treatment is rising. Attention for long-term side effects is therefore of growing importance. This attention is also stimulated by studies in survivors of childhood cancer which have shown a worrisome high incidence of late cardiovascular toxicity after anthracycline therapy.^{3,4,33} In addition, the incidence of cardiovascular toxicity in these survivors is increasing with longer follow-up duration. The same may apply for adults, however, the number of well-designed studies on the risk of late cardiovascular toxicity in adults is limited.

Most reports on this issue are hampered by the insensitive end point cardiac mortality and with limited follow-up duration, so the full impact of the problem may be underestimated.

Cardiotoxicity not only refers to patients that already have developed clinical symptoms of cardiotoxicity such as heart failure or who have died from it, but also to subclinical cardiotoxicity that may become clinical manifest in later stage. Cardiotoxicity of regimens should therefore be determined through baseline evaluation (before chemotherapy) and subsequent serial measurements of the cardiac function, for example by LVEF measurements by radionuclide ventriculography or echocardiography. A decrease of LVEF after cessation of chemotherapy to a value below 50% or a significant decrease that remains within the normal range is often not accompanied by clinical symptoms, but may result in congestive heart failure in the long-term, although the predictive value of such decreases in LVEF is not known. More studies are required with expanded follow-up and better end points determined by cardiac monitoring to get accurate incidence figures.

Also for new and future chemotherapy regimens the evaluation of cardiovascular toxicity should not be restricted to the short-term. This especially applies for a number of new developments in the treatment of breast cancer. Increased cardiotoxicity has been reported after new promising agents including the taxoids,^{192,193} and the monoclonal antibody trastuzumab,¹⁹⁴ especially when combined with anthracyclines. Currently these agents are used and evaluated in the treatment of metastatic breast cancer, but in the future their application may be extended to the adjuvant use, making the long-term risk of late cardiotoxicity more important.

Minimizing the risk of late cardiovascular toxicity is therefore an important but difficult challenge. Omitting the causative agent or reducing its dosage is likely to be accompanied by less treatment efficacy. In the treatment of testicular cancer, for example, attempts have been made to limit pulmonary and vascular toxicity by omitting the agent bleomycin. However, this resulted in less tumor efficacy and so bleomycin is still used despite its toxic effects.¹⁹⁵ Alternative ways to circumvent toxicity are currently being investigated. Less toxic administration forms, such as anthracyclines incorporated into liposomes, may help to reduce toxicity.¹⁹⁶ Also less toxic analogues have been developed, such as epirubicin for doxorubicin and carboplatin for cisplatin. However, their use also may result in less tumor efficacy, as was recently shown for carboplatin in the treatment of testicular cancer.¹⁹⁷ The application of chemoprotectants such as dexrazoxane or amifostine during chemotherapy is a promising way to limit direct cardiovascular toxicity, but their use needs further evaluation.¹⁹⁸

In the case of anthracycline-induced cardiotoxicity, guidelines have been developed by Schwartz et al. for serial cardiac monitoring during anthracycline chemotherapy that allow an optimal cumulative dose for an effective anti tumor treatment, with a minimal risk of inducing late cardiotoxicity.¹⁹⁹ These guidelines include: (1) a baseline radionuclide ventriculography determined LVEF measurement in all patients; (2) the next measurement at 300 mg/m² and again at 400 mg/m² in patients with additional risk factors or at 450 mg/m² in the absence of risk factors; (3) a repeated measurement prior to each subsequent dose beyond 450 mg/m²; (4) discontinuation of chemotherapy if LVEF declines by more than 10% (absolute EF units) to a EF value

of less than 50%. It is however, not known whether late onset cardiotoxicity that may occur several years after completion of therapy will be prevented using these guidelines. Monitoring cardiac function is particularly relevant in patients who have an increased risk of developing chemotherapy-induced cardiotoxicity, namely patients with pre-existent cardiovascular disease and patients treated with high doses of potential cardiotoxic chemotherapy.

How often and by which detection techniques and parameters patients should best be measured after completion of potential cardiotoxic chemotherapy to prevent late onset cardiotoxicity has not yet been elucidated. The Cardiology Committee of the Childrens Cancer Study Group has proposed that those patients who have received a high anthracycline dose (more than 450 mg/m²) and patients with additional risk factors at 3-6 months, and 12 months after completion of therapy should be monitored.²⁰⁰ When cardiac function remains normal during the first year they further suggest that cardiac function should be monitored every 2 to 3 years, but more frequently when cardiac abnormalities have been detected.²⁰⁰

Whether such guidelines developed for children and adolescents will be useful and cost-effective for adult patients has not been evaluated yet. Also the detection technique of choice for monitoring the cardiac function after completion of chemotherapy is under discussion. Although for adults the resting LVEF measurement by radionuclide ventriculography or by echocardiography is the most widely applied technique, other techniques such as LVEF measurement after dobutamine or physical stress, and the determination of diastolic dysfunction parameters, may help to identify cardiotoxicity in a subclinical stage.²⁰¹ From studies performed in patients with decreased ischemic-related cardiac function it has become evident that intervention with inhibitors of angiotensin-converting enzyme and possibly β -adrenergic blockers, slows down the deterioration of the cardiac function.^{202,203} To date no such studies have been performed in asymptomatic patients with chemotherapy-related left ventricular dysfunction. However, it seems likely that the same benefit may be achieved in this patient group, although studies supporting this are not available yet.

Indirect toxic effects on the cardiovascular system such as the occurrence of an unfavorable lipid profile as a consequence of gonadal toxicity might be limited by hormonal replacement. Particularly in female non-breast cancer patients who have developed premature menopause, estrogen replacement therapy may prevent or revert unfavorable changes in the lipid profile, so averting the occurrence of premature cardiovascular morbidity.²⁰⁴

It is the duty of oncologists to be alert to possible late cardiovascular toxicity. In the follow-up after treatment patients should be asked regularly about cardiac complaints so that evaluation by a cardiologist could be requested. Finally, the risk of cardiovascular morbidity could be minimized by monitoring and treatment of cardiovascular risk factors such as dislipidemia and hypertension, in order to maximize quality of life after chemotherapy.

CHAPTER 3

Detection of anthracycline-induced cardiotoxicity

*M.T. Meinardi¹, W.T.A. van der Graaf¹, D.J. van Veldhuisen², J.A. Gietema¹,
E.G.E. de Vries¹, D.Th. Sleijfer¹*

Departments of Medical Oncology¹ and Cardiology²,
University Hospital Groningen, The Netherlands.

Cancer Treatment Reviews 1999; 25: 237-247

SUMMARY

The use of anthracyclines, a group of potent anti-cancer agents incorporated into the treatment of a wide variety of solid and hematological tumors, is limited by its cardiotoxicity that can result in congestive heart failure (CHF). The best method to detect cardiotoxicity in an early stage in order to prevent severe deterioration, is still an unsolved problem. Although endomyocardial biopsy is considered to be the most sensitive and specific test for this purpose, its use is limited by its invasiveness. In daily practice, oncologists make use of parameters of systolic function (left ventricular ejection fraction or fractional shortening) to detect cardiotoxicity, but these methods are not able to identify cardiotoxicity at an early stage.

Based on increasing knowledge into the pathophysiology of anthracycline-induced cardiotoxicity and heart failure in general, new methods including the determination of diastolic function parameters, anti-myosin scintigraphy, assessment of heart rate variability, and the determination of biochemical markers have been proposed to identify patients at risk of the development of CHF in an early stage. However, most of these newer methods have not yet been adequately evaluated to allow them to be recommended for use in routine clinical practice.

INTRODUCTION

Since their introduction in the late 1960s, anthracyclines such as doxorubicin and epirubicin have been successfully used in the treatment of a wide variety of hematopoietic and solid tumors. However, their use is limited by the occurrence of cardiotoxicity, which may result in congestive heart failure (CHF). This cardiotoxicity can become clinically overt shortly after anthracycline treatment, but also many years later.^{4,205} The magnitude of this problem is rising as a result of the growing number of long-term cancer survivors who have been treated with anthracycline containing chemotherapy.²⁰⁶ Moreover, the use of anthracyclines has been increased, particularly as adjuvant therapy.

To detect cardiac dysfunction in patients who are treated with anthracyclines, regular monitoring of the heart function during treatment is important. Identification of cardiac damage may lead to discontinuation or to a dose reduction of the anthracyclines in order to prevent further cardiac deterioration. After completion of chemotherapy, detection of cardiac dysfunction is also of relevance since this might lead to timely medical intervention aiming at improvement of the cardiac prognosis. Furthermore, in clinical studies of new anthracycline analogues, the cardiac function needs to be monitored. For this monitoring many different techniques are available. However, because each technique has specific limitations, agreement on the use of them is lacking. In this review we present an overview of the different methods that currently are used as either standard or investigational to detect anthracycline-induced cardiotoxicity. After describing the different manifestations, the risk factors and the pathogenesis of anthracycline-induced cardiotoxicity, the detection methods will be discussed in terms of weighing their advantages, disadvantages and usefulness in

medical practice.

Clinical manifestations and risk factors for anthracycline-induced cardiotoxicity

Anthracycline-induced cardiotoxicity is divided into acute and chronic cardiotoxicity. Acute cardiotoxicity occurs during or immediately after anthracycline administration and consists mainly of electrophysiological abnormalities and arrhythmias.²⁰⁷ In the majority of cases acute cardiotoxicity is transient and does not result in chronic cardiac dysfunction.²⁰⁸ Occasionally, severe cases of acute left ventricular dysfunction and of a pericarditis-myocarditis syndrome have been reported.^{205,209} However, with the current anthracycline dose regimens, this type of cardiotoxicity has become a relatively rare clinical issue and therefore will not be discussed further in this review.

Clinically more relevant is the chronic form that presents as ventricular dysfunction which can result in CHF. Chronic cardiotoxicity may occur weeks or up to many years after anthracycline therapy,³ but most cases with severe cardiac dysfunction are diagnosed within one year.²

Several risk factors have been identified that contribute to the development of chronic cardiotoxicity. The most important risk factor is the total cumulative dose of the anthracycline given. Lefrak et al. reported a 30% incidence of CHF in patients treated with cumulative doses of doxorubicin above 550 mg/m² body surface area.²¹⁰ Von Hoff et al. found that the risk of doxorubicin-induced CHF was less than 10% when a cumulative dose below 550 mg/m² had been administered while the risk increased significantly above this dose.² These observations have led 550 mg/m² doxorubicin being considered to be the maximum 'safe' cumulative dose. For epirubicin, a doxorubicin analogue with less cardiotoxicity, a comparable threshold has not been precisely defined, but is considered to be about 900-1000 mg/m².²¹¹⁻²¹³ However, a wide variation exists in the individual cardiac sensitivity to anthracyclines. Schwartz et al. found that in 1487 cancer patients who had been treated with anthracyclines, the total cumulative dosages of doxorubicin that caused CHF varied widely from 75 to 1095 mg/m².¹⁹⁹ There are several reports of patients who have tolerated doses in excess of 1000 mg/m², up to even 5000 mg/m² doxorubicin without developing CHF.^{2,205,214} This variation in individual sensitivity implies that an arbitrary dose limitation of 550 mg/m² for doxorubicin in order to avoid cardiotoxicity may deprive some patients who safely could receive a much higher dose of doxorubicin of the maximum therapeutically benefit from the drug.

Besides the cumulative dose of the anthracycline, several other factors can contribute to the development of chronic cardiotoxicity. These include high serum peak levels of anthracyclines,^{215,216} mediastinal radiation,¹³⁰ a young age or advanced age at the time of treatment,^{2,217,218} pre-existing heart disease and hypertension² and female sex.^{5,219} Also combination treatment with other anti-cancer drugs such as cyclophosphamide¹¹¹ and mitomycin-C²²⁰ may possibly increase the risk of anthracycline cardiotoxicity.

Pathogenesis

The pathogenetic mechanism of anthracycline-induced cardiac damage is not fully understood. Several mechanisms play a role, of which the anthracycline-mediated production of free oxygen radicals appears to be the most important.²²¹⁻²²⁴ These free radicals lead to destruction of the mitochondria so causing myocyte death. Also calcium overload in the myocyte appears to be involved, caused by an anthracycline-induced activation of the calcium-release channel.^{204,225-227} Furthermore, anthracyclines appear to have a direct toxic effect on the adrenergic function of the myocardium.²²⁸⁻²³¹ These mechanisms disturb the contractility of the myocardium. To compensate for a decrease in cardiac output, several adaptive mechanisms are activated to maintain the pump function of the heart, probably similar to those that have been identified in other forms of heart failure, such as an increase in heart rate, an increase in contractility, an increase in preload and an increase in the number of contractile elements.²³² These adjustments are accomplished by an increase in activity of two interrelated neurohormonal / autocrine-paracrine systems, the adrenergic and the renin-angiotensin-aldosterone systems. As a consequence, the wall stress (afterload) increases and the ventricles dilate. This leads to activation of natriuretic peptides, excreted by the atria (Atrial Natriuretic Peptide) and ventricles (Brain Natriuretic Peptide) that counter-regulate the effects of the adrenergic and the renin-angiotensin-aldosterone systems.²³³ At the mechanistic level, it is thought that first the diastolic function of the heart is disturbed, before the systolic function deteriorates.^{22,23,234} When the systolic function diminishes severely, CHF can arise.

In most adult patients, the cardiac changes follow a pattern of chronic dilated cardiomyopathy.^{210,235} In young cancer survivors cured by anthracyclines, a reduced left ventricular mass and thinning of left ventricular walls have been found,²⁴ which in some patients results in a restrictive cardiomyopathy.³³

Detection of anthracycline cardiotoxicity

The detection of anthracycline cardiotoxicity is useful during chemotherapy, during follow-up and in investigational studies. The goal of monitoring during chemotherapy is to administer the highest dose of anthracyclines without causing severe cardiac damage.

The purpose of monitoring after completion of chemotherapy is identification of cardiac damage at an early preclinical stage in order to prevent further deterioration by timely intervention. Finally, monitoring the cardiac function is of importance in clinical studies, in which the cardiotoxicity of new anthracycline analogues, liposomal doxorubicin¹⁹⁶ or the usefulness of cardioprotective agents such as dexrazoxane^{236,237} and probucol²³⁸ is evaluated. Different techniques are available to detect anthracycline cardiotoxicity, each having its advantages and limitations.

Chest radiograph

Only in a late stage of the disease, when clinical signs of CHF are present, a chest ra-

diograph can reveal cardiomegaly or pulmonary edema. However, a chest radiograph is not capable to detect early signs of anthracycline cardiotoxicity.²³⁹ Therefore, the usefulness of chest radiographs in the detection of anthracycline cardiotoxicity is strictly limited.

Electrocardiography (ECG)

The 12-lead ECG is a non-invasive, readily available technique by which different cardiac abnormalities can be detected. During or immediately after administration of anthracyclines ECG abnormalities are encountered in approximately 11 % of patients treated (range 0-41 %).^{240,241} These electrocardiographic abnormalities include non-specific ST-segment and T-wave abnormalities and arrhythmias. These findings usually are transient and do not reflect nor predict the development of chronic cardiotoxicity.^{2,208,210}

Comparison of summations of QRS voltage have been used historically to indicate chronic cardiotoxicity.^{220,242} The decrease of QRS voltage is generally a late phenomenon that coincides with the onset of CHF.²⁴¹ In addition, a decrease in QRS voltage can also be observed in patients who develop pleural or pericardial effusion as a result of tumor progression without CHF, indicating that this ECG-parameter has a low specificity for anthracycline cardiotoxicity.

A prolonged QTc interval has also been suggested to be an early sign of ventricular dysfunction.^{24,243,244} However, Ferrari et al. showed in a follow-up study that prolongation of the QTc (more than 0.45 sec) was transient in most of the patients. Moreover, no correlations between prolonged QTc and functional echocardiographic alterations were found.²⁴⁵

It is concluded that the ECG can show several abnormalities associated with anthracycline administration, but given the low sensitivity and the low specificity its value for the detection of chronic anthracycline-induced cardiotoxicity is limited.^{31,246}

Multigated Radionuclide Angiography (MUGA)

MUGA is a non-invasive technique that makes use of intravenously injected radionuclides (⁹⁹Technetium) that bind to erythrocytes and enable the cardiac pool to be visualized with a γ -camera. This technique provides assessment of various cardiac indices such as the left ventricular ejection fraction (LVEF) for systolic function and the peak filling rate for diastolic function. Measurement of LVEF is widely used, particularly in adults and its value for this purpose has been investigated thoroughly.^{21,199,239,247-249} Serial LVEF measurements have been shown to be useful in clinical practice. Alexander et al. found that a decline in LVEF by at least 15% to a final value of less than 45% preceded the development of CHF.²⁴⁷ Based on their experiences, Schwartz et al. developed guidelines that included: 1) a baseline LVEF measurement in all patients; 2) the next measurement at 300 mg/m² and again at 400 mg/m² in patients with additional risk factors or at 450 mg/m² in the absence of risk factors; 3) a repeated measurement prior to each subsequent dose beyond 450 mg/m²; 4) discontinuation of anthracyclines if LVEF declines by more than 10% (absolute EF units) to a EF value of less than 50%.¹⁹⁹ In a retrospective analysis among 282 patients, they found, in the patient group in which these guidelines were used precisely, a 4-fold reduction in the risk of

CHF compared to a group in which these guidelines were not followed.

However, for the early detection of anthracycline-induced myocardial damage the LVEF measurement is not very sensitive because the decrease occurs late in the pathological process from cellular myocardial damage to CHF. McKillop et al. found that the resting LVEF determination had a sensitivity of only 53% and a specificity of 75% compared to abnormalities found in myocardial biopsy specimens.²¹ In addition, LVEF determination is influenced by changes in preload and afterload as may occur in conditions such as fever or septicemia or by volume infusions. Also alterations in sympathetic tone, for example due to anemia, affect the result of the LVEF determination.

To increase its sensitivity, LVEF measured during stress (maximal exercise or by dobutamin infusions) has been proposed. By stress testing, the heart is forced to increase the cardiac output, which in a normal healthy heart leads to an increase with at least 5% absolute EF units.²⁵⁰ Failure to increase the LVEF by this amount indicates a decreased cardiac reserve. Patients with a low resting LVEF, but with sufficient cardiac reserve (a rise in LVEF of more than 5%) may be able to tolerate additional anthracycline therapy.²⁵¹ In the previous mentioned study of McKillop et al. the exercise LVEF determinations increased the sensitivity to detect cardiotoxicity to 89% (compared to 53% for resting LVEF determinations).²¹ However, the specificity was found to be decreased to 41% (compared to 75% for resting determinations). Furthermore, the applicability of exercise testing during chemotherapy may be hampered by the poor physical condition of many cancer patients.

Since diastolic dysfunction generally precedes systolic dysfunction in other forms of heart failure,^{252,253} studies have also focussed on the usefulness of diastolic function parameters including the peak filling rate, the rapid filling velocity and slow filling velocity. Lee et al. found a normal LVEF of 55% or more before and after doxorubicin treatment, but the mean rapid filling velocity and the slow filling velocity were both significantly reduced.²⁵⁴ Similar findings have been reported by Ganz et al. who also found that diastolic dysfunction, as shown by a decreased peak filling rate, preceded a decrease in LVEF in patients who eventually developed anthracycline-induced CHF.²³⁴ However, Cottin et al. found simultaneous impairment of the LVEF and the peak filling rate.²⁵⁵ Furthermore, it should be realized that diastolic parameters are also influenced by changes in hemodynamics, particularly changes in heart rate and blood pressure.²⁵⁶

In conclusion, MUGA is one of the most important techniques to detect anthracycline-induced cardiotoxicity in clinical practice. However, its limited sensitivity and the influence of changes in loading conditions should be taken into account. Although LVEF determinations during stress may increase the sensitivity compared to resting determinations, this is at the expense of a decrease in specificity. Probably the determination of the diastolic function reveals cardiotoxicity in an earlier stage.

Echocardiography

Echocardiography is one of the most widely used tools in cardiology that allows non-invasive evaluation of cardiac function. To detect anthracycline-induced cardiotoxicity,

echocardiography is used frequently, particularly in pediatric oncology. Various parameters of systolic function, diastolic function, anatomic dimensions and afterload can be assessed. Although the LVEF can also be measured by echocardiography, most frequently the fractional shortening (FS) is used.^{21,257-260} In pediatric oncology, a FS below 28-30% is considered to reflect systolic dysfunction. The Cardiology Committee of the Children's Cancer Study Group has proposed guidelines for modification of anthracycline-based chemotherapy using the FS.²⁰⁰ The authors suggest that a drop of more than 10 percentile units compared to the initial values, or a FS below 29% is indicative of significant deterioration of the cardiac function and a reason to withhold further anthracycline treatment. By using their guidelines, they reported only three (0.4%) of 766 patients who developed CHF. In addition, 43 patients were able to tolerate more than 550 mg/m² doxorubicin. Since FS is a systolic parameter the same restrictions as mentioned for LVEF determination by MUGA, apply for its determination of FS.

To examine the myocardial contractility more directly, the stress velocity index has been proposed.³³ This more complicated technique requires measurement of a carotid pulse tracing, a phonocardiogram and blood pressure simultaneous with the echocardiographic recording. The index incorporates measurements of contractility, afterload and preload, allowing their independent contribution to overall left ventricular performance. Lipshultz et al. reported that in 23% of long-term survivors of childhood cancer the stress velocity index was decreased.³³ In a later study, a strong association between the decrease in stress velocity index and the total cumulative anthracycline dose was found.⁵ However, this technique has not been widely accepted because of its technical complexity.

Since echocardiographic analysis of transmitral Doppler flow patterns to determine LV diastolic function has been shown to provide useful information about pathogenesis and prognosis in various types of heart disease,^{261,262} its value for the detection of anthracycline-induced cardiotoxicity has also been investigated.^{22,255} Parameters used to assess the diastolic function include the isovolumic relaxation time (IVRT) and the early peak flow velocity / atrial flow velocity (E/A) ratio.²⁶³ Marchandise et al. found in a prospective study in 19 adult patients no post-treatment LV systolic dysfunction.²⁵⁵ However, the mean IVRT was prolonged by 22% and the E/A ratio was decreased by 20% compared to baseline determinations, indicating diastolic dysfunction. Stoddard et al. also reported an increase in the mean IVRT from 66 to 84 ms in a prospective study in 26 anthracycline-treated adults. This increase could be found after cumulative doxorubicin doses as low as 100 mg/m² and preceded a decline in systolic function.²² We also found in a cross-sectional study in breast cancer patients, who had been treated with anthracyclines, a disturbed diastolic function in 50% of the patients, despite a normal systolic function (LVEF > 50%).²⁶⁴

On the other hand, Ewer et al. were unable to find changes in any of the measured parameters of diastolic function in spite of a significant decline in systolic function as measured with FS.²⁶⁵ In addition, Sung et al. found abnormal diastolic parameters in children who had been treated with anthracyclines as well as in children who had been treated with non-anthracycline containing chemotherapy.²⁶⁶

With increasing follow-up of long-term survivors of childhood cancer and with more precise echocardiographic measurements, it has become clear that there is a high incidence of cardiac abnormalities among these patients. These changes include thinning of the left ventricular posterior wall (LVPW) and reduction of the left ventricular mass (calculated by echocardiographically determined anatomic dimensions) and increases in the afterload. Lipshultz et al. noted a lower LVPW indexed for body surface area (LVPWi) in long-term survivors compared to normal controls.³³ Furthermore, the afterload (measured by the end-systolic wall stress) was increased compared to controls and was found to be more increased in patients who were treated with higher cumulative doses of anthracyclines. Similar findings have been reported by others.^{24,259} Whether these cardiac abnormalities eventually lead to CHF is uncertain, but are a matter of great concern.

Finally, echocardiography during stress tests has been investigated to unmask subclinical cardiotoxicity. Both exercise and dobutamin stress tests have been used for this purpose. Yeung et al. using exercise echocardiography, found in 19 asymptomatic children, treated with anthracyclines up to 6 years before, a normal rest FS but an increase in FS of only 3% compared with a mean increase of 23% in a group of patients treated with non-anthracycline containing chemotherapy.²⁶⁷ Others have reported similar findings.²⁶⁸

By using dobutamine stress echocardiography Klewer et al. analyzed the cardiac status of 21 long-term survivors of childhood cancer treated with anthracyclines.²⁶⁹ Data were compared with 12 age-matched healthy controls. Differences between the patients and the controls of the end-systolic LVPW dimensions were much more evident during stress compared to resting determinations. In particular, FS and end-systolic left ventricular wall stress were abnormal only during dobutamine stimulation.

In conclusion, for monitoring the cardiac function during chemotherapy in order to adapt anthracycline doses, echocardiographic parameters of the systolic function (LVEF or FS) can be used, but their limited sensitivity to detect early cardiac damage should be realized.

The value of FS in adults has been evaluated inadequately and can therefore not be recommended. By using diastolic parameters, anthracycline cardiotoxicity appears to be detected in an earlier stage, but more extensive evaluation is still needed to determine its exact prognostic value. Other parameters, such as the stress-velocity index, the thinning of the LVPW and the end-systolic wall stress also need further prospective evaluation.

Heart Rate Variability (HRV)

HRV is the fluctuation in the interval between consecutive heartbeats, which is controlled by the autonomic nervous system. Normal subjects are able to modulate the heart rate in a wide range of different levels which can be modified very rapidly. HRV can be assessed by 'spectral analysis' and 'time domain analysis' of the fluctuations in heart rate, calculated from 24-hour ambulatory ECG recordings by using specific computer software. Various studies have shown that HRV is impaired in patients with heart failure.²⁷⁰⁻²⁷² In addition, the degree of impaired HRV appears to have a prognos-

tic role.^{273,274}

The value of HRV analysis for the detection of anthracycline-induced cardiotoxicity has been evaluated in a few studies. Postma et al. tested 31 young patients for late cardiotoxicity (follow-up range 2.3-14.1 years (median 8.9 years)) with several techniques (MUGA, echocardiography) and also with HRV analysis.²⁴ Correlation between the anthracycline dose and echocardiographic and MUGA parameters were not found, but HRV analysis revealed a significantly impaired HRV in the patients who received more than 400 mg/m² doxorubicin compared to those who received less than 400 mg/m², suggesting that HRV could be a sensitive indicator for cardiotoxicity.²⁴ We recently found in breast cancer patients who had been treated with anthracyclines and high-dose chemotherapy, a significantly impaired HRV compared to healthy age-matched females.²⁶⁴ Particularly parameters reflecting the parasympathetic activity (rMSSD, pNN50) were impaired, which is also the case in early stages of other forms of heart failure.²⁷²

In conclusion, HRV analysis appears to be a sensitive test for the detection of anthracycline cardiotoxicity but further study is needed to determine the ultimate predictive value and specificity. Until then, HRV is of potential utility in examining mechanisms but it is still of very limited clinical utility in the evaluation of individual patients.

Biochemical markers

Natriuretic peptides

The natriuretic peptides Atrial Natriuretic Peptide (ANP), and Brain Natriuretic Peptide (BNP) are currently evaluated for their usefulness in the detection of anthracycline cardiotoxicity. ANP is secreted mainly by the atria in response to increased stretch as a result of an increased left-atrial pressure, as is N-terminal ANP, the inactive type of ANP that is less rapidly cleared. BNP is produced mainly by the ventricles in response to ventricular dilatation and increased wall stress.²⁷⁵ Plasma levels of both peptides are elevated in both symptomatic and asymptomatic patients with left ventricular dysfunction from other causes.²⁷⁵ Their applicability as a marker for anthracycline-induced cardiotoxicity has only been investigated in a few small studies. Bauch et al. measured plasma ANP-levels prospectively in 19 children treated with anthracyclines. Two out of six patients with elevated plasma ANP-levels 3 weeks after completion of the anthracycline treatment developed CHF, while a decline in LVEF determined by MUGA could not be established.²⁷⁶ In contrast, Yamashita et al. found in 30 cancer patients treated with anthracyclines that elevated plasma levels of ANP were a late phenomenon that coincided with the development of CHF.²⁷⁷

A follow-up study by Tikanoja et al. showed a significant increase in the serum N-terminal ANP-levels in patients after anthracycline chemotherapy compared to age-matched controls (follow-up 0.9-13 years).²⁷⁸ Suzuki et al. found a significant increase in BNP-levels during anthracycline-based chemotherapy, however, in most of these patients these elevations were transient. On the other hand, two out of three patients with a persistently elevated BNP-level subsequently died as a result of circulatory fail-

ure.²⁷⁹

Hence, ANP and BNP are potential biochemical markers to detect anthracycline-induced cardiotoxicity, but at present they have been evaluated inadequately both during treatment and during follow-up.

Cardiac troponin

Other biochemical markers that have been investigated recently for their use in the diagnosis of anthracycline-induced cardiotoxicity are the cardiac troponins-T and I. These thin-filament contractile proteins are present in high concentrations in the myocardium and are released rapidly into the circulation after myocardial injury such as myocardial infarction or acute myocarditis.²⁸⁰

Lipshultz et al. found elevated cardiac troponin-T serum levels in 15 children who were treated with doxorubicin therapy. They found that the degree of elevation predicted left ventricular dilatation and wall thinning nine months later, suggesting that an elevated cardiac troponin-T level may predict subsequent subclinical and clinical cardiac morbidity.²⁸¹

Missov et al. also reported elevated cardiac troponin-I levels in 13 anthracycline treated patients, compared to both anthracycline naive patients and healthy controls.²⁸²

Cardiac troponins are potentially useful markers for the early detection of anthracycline-induced cardiotoxicity, but they have been inadequately investigated.

Endothelin-1

Endothelin-1 is a potent vasoconstrictor peptide, synthesized in the vasculature and the myocardium by endothelial cells and ventricular myocytes. Plasma endothelin-1 levels are elevated in patients with severe heart failure from different causes and a correlation exists between the plasma level and the severity of heart failure. The exact role of endothelin-1 in the pathophysiology of heart failure is not fully understood.²⁸³

Yamashita et al. measured changes in plasma endothelin-1 during doxorubicin treatment in 30 cancer patients, in whom the cardiac function was also evaluated by echocardiography. The plasma levels of endothelin-1 rose progressively in five patients. Later on, two of these 5 patients developed clinically overt CHF after chemotherapy, while the FS and LVEF showed no abnormalities until the development of CHF.²⁷⁷ This suggests a potential role for the determination of plasma endothelin-1 levels to predict anthracycline-induced cardiotoxicity.

Radionuclide imaging tests

Besides MUGA, other radionuclide imaging tests have been proposed to detect anthracycline cardiotoxicity at the cellular level.

¹¹¹Indium-Antimyosin heart scintigraphy

Antimyosin scintigraphy makes use of the myocardial uptake of intravenously injected ¹¹¹Indium-antimyosin antibodies. Binding of this antibody to intracellular myosin occurs only when the sarcolemma is disrupted and the cell has been irreversibly dam-

aged.²⁸⁴⁻²⁸⁷ Carrio et al. studied in 36 cancer patients, the applicability of antimyosin scintigraphy.²⁸⁵ Antimyosin scans and ejection fraction measurements were performed before, during and at the end of doxorubicin treatment. Antimyosin uptake was quantified by a heart-to-lung ratio. While none of the patients had an elevated antimyosin uptake (mean ratio 1.40 ± 0.06) before chemotherapy, an increased uptake was observed during and at the end of chemotherapy (mean ratio of 2.02 ± 0.3). Patients with more intense antimyosin uptake during chemotherapy tended to be those with more severe functional impairment measured by a decrease in LVEF. Also others suggest that morphological damage in the myocytes can be detected by antimyosin scans before the systolic function deteriorates.^{288,289}

¹²³Iodine-Metaiodobenzylguanidine (MIBG) heart scintigraphy

MIBG behaves similarly to noradrenaline and is taken up by the myocardium.²⁹⁰ MIBG has been found to be a suitable marker to study myocardial adrenergic innervation and neuron injury.²⁹¹ In patients with heart failure MIBG studies have shown disturbances in adrenergic neurone activation.^{292,293} MIBG scintigraphy has also been proposed to assess impairment of the adrenergic function due to anthracycline cardiotoxicity.^{285,294-297} For instance, Valdes Olmos et al. found in 21 patients treated with anthracyclines, that the cardiac adrenergic derangement occurred before the development of systolic dysfunction measured by LVEF determinations. Moreover, impaired MIBG uptake tended to correlate with the cumulative doses of anthracyclines.²⁹⁵ Carrio et al. reported similar findings.²⁸⁵

Radionuclide imaging techniques of the heart using anti-myosin-antibodies and MIBG are potentially useful techniques, which can detect anthracycline cardiotoxicity in an early stage. However, further prospective evaluation in larger trials is needed to clarify the specificity and the prognostic value of these both techniques. In addition, both tests are not available in many centers and require radioactive nuclides which is a disadvantage for frequent screening.

Endomyocardial biopsy

Endomyocardial biopsy obtained from the right ventricle during heart catheterization is considered to be the most sensitive and specific test for the detection of anthracycline-induced cardiotoxicity. Electron microscopic examination of the biopsy specimens reveals characteristic changes, including extensive depletion of myofibrillar bundles, myofibrillar lysis, distortion and disruption of the Z-lines, mitochondrial swelling and particularly swelling and disruption of the sarcoplasmic reticulum, leading to intramyocyte vacuolisation.²⁹⁸⁻³⁰¹ In patients with mild toxicity this damage is patchy, but with increasing toxicity this damage is found more extensively throughout the heart with progression to myocyte necrosis and myocyte loss. Several studies in which endomyocardial biopsy was compared with other detection techniques such as MUGA, echocardiography and ECG-recordings have shown that endomyocardial biopsy examination can detect cardiotoxicity before any sign of LV dysfunction is detected.^{298,299,301}

Billingham et al. developed a standardized grading scale for the degree of dam-

age.²⁹⁸ The grade of injury can be expressed on a scale ranging from 1 to 3: biopsy samples in which less than 5% of cells show changes typical of doxorubicin injury are graded 1, and those with changes in 5-15%, 16-25%, 26-35%, and more than 35% of cells are graded 1.5, 2, 2.5, and 3 respectively. This grading scale correlates with the cumulative anthracycline dose and has a predictive value for the development of CHF. Although this detection technique is considered to be the most sensitive for chronic cardiotoxicity, sampling errors can occur because of the scattered cardiac damage.³⁰²

In conclusion, endomyocardial biopsy examination is considered the most sensitive and specific method for the detection of chronic anthracycline-induced cardiotoxicity and prediction of cardiac dysfunction. Unfortunately, the use of endomyocardial biopsy for routine monitoring is limited by its invasiveness, and the lack of expertise in obtaining and interpreting these biopsy specimens. Therefore, this test is mainly useful in those situations in which there is doubt about the cause of cardiac dysfunction in a patient treated with anthracyclines.

DISCUSSION

Although cardiotoxicity is a well-recognized side-effect of anthracycline treatment, this problem remains a clinical problem, especially because of the increasing number of long-term cancer survivors and because of the increasing number of patients treated with adjuvant (anthracycline-based) chemotherapy. To prevent cardiotoxicity, several different alternative administration schedules,²¹⁶ the use of cardioprotective agents such as dexrazoxane³⁰³ and the use of liposomal anthracyclines that possibly cause less cardiotoxicity¹⁹⁶ have been investigated. Because the success of these strategies is still uncertain and limited, regular monitoring of cardiac function is and will be essential to prevent severe cardiac deterioration.

Monitoring cardiotoxicity is useful during chemotherapy, during follow-up and in investigational studies in which new anthracycline analogues are evaluated. The gold standard is the endomyocardial biopsy but its use in medical practice is limited by its invasiveness. Although many different new techniques have been proposed to guide anthracycline therapy, most of them have been inadequately investigated. Therefore, oncologists in daily medical practice are still thrown on the conventional determinations of the left ventricular systolic function by MUGA or by echocardiography, although the measurement of the systolic function clearly has its limitations as has been discussed.

For guiding anthracycline therapy in adults the guidelines as described by Schwartz et al can be used.¹⁹⁹ However, one must be sure that a decline in LVEF is not caused by changes in patients' hemodynamics or by alterations in the serum hemoglobin level.

Young patients need to be monitored more frequently. The Committee of the Children Cancer Study Group has developed guidelines using various detection techniques including echocardiography and MUGA.²⁰⁰ In the future, newer methods such as the determination of diastolic parameters, analysis of HRV or the use of radionuclide imaging tests may be of (additional) help to identify cardiotoxicity in an earlier

stage and allow an optimal cumulative anthracycline dosage to be adapted on an individual basis. However, this first requires evaluation in large patient groups.

During the follow-up, cardiotoxicity may be evaluated by determinations of systolic function. Additionally, assessment of the diastolic function can be used. Patients who have received a high anthracycline dose (more than 450 mg/m²) and patients with additional risk factors should be monitored at 3 to 6 months, and 12 months after completion of therapy. If the cardiac function remains normal during the first year further monitoring should be performed every 2 to 3 years, but more frequently when cardiac abnormalities have been detected.²⁰⁰ In those patients in whom deterioration of the systolic function is found, stress testing may be useful to analyze the cardiac reserve. Continuous monitoring could facilitate early therapeutic intervention that may prevent further cardiac deterioration. In patients with mild left-ventricular systolic dysfunction from other causes, treatment with angiotensin-converting enzyme inhibitors has been shown to decrease morbidity and mortality.²⁰² Early treatment of patients with subclinical anthracycline-induced cardiotoxicity with these drugs might also be beneficial, but has still to be proven, preferably in randomized clinical trials.

Finally, patients treated with investigational anthracycline analogues with unknown cardiotoxicity should be monitored very carefully. Only patients without pre-existing heart disease and with a normal ECG should be eligible for such trials to minimize the risk of severe cardiac deterioration.² At baseline a LVEF measurement with MUGA or echocardiography should be performed. Before each course a subsequent LVEF determination should be done. To be able to make a comparison with conventional anthracyclines, it is essential to continue monitoring after completion of the therapy. However, since patients who participate in such trials usually have a limited life expectancy, signs of subclinical cardiac toxicity should be sought more actively. Therefore, in these settings, besides the determination of the systolic function, the diastolic function of the left ventricle should be evaluated either by echocardiography or by MUGA.

For the future, further evaluation of new parameters of early cardiac damage is of relevance to define patients who could benefit from early intervention measures to prevent or delay the development of anthracycline-induced congestive heart failure.

CHAPTER 4

Cardiovascular morbidity in long-term survivors of metastatic testicular cancer

*M.T. Meinardi¹, J.A. Gietema¹, W.T.A. van der Graaf¹, D.J. van Veldhuisen²,
M.A. Runne¹, W.J. Sluiter³, E.G.E. de Vries¹, P.H.B. Willemse¹, N.H. Mulder¹,
M.P. van den Berg², H. Schraffordt Koops⁴, D.Th. Sleijfer¹*

Departments of Medical Oncology¹, Cardiology², Endocrinology³, and
Surgical Oncology⁴, University Hospital Groningen, The Netherlands

Journal of Clinical Oncology 2000;18(8):1725-32

SUMMARY

Purpose. To determine whether long-term survivors of metastatic testicular cancer have an have an increased risk of cardiovascular morbidity more than 10 years after chemotherapy.

Patients and Methods. Eighty-seven patients treated with cisplatin containing chemotherapy before 1987 who were in remission for at least 10 years and whose ages were ≤ 50 years at the time of analysis were evaluated for the occurrence of cardiovascular events. Sixty-two of 87 patients were additionally evaluated for cardiac damage and cardiovascular risk factors. Their cardiovascular risk profile was compared with that of 40 patients with comparable age and follow-up duration treated with orchidectomy only for stage I disease.

Results. Major cardiac events were found in five (6%) of the 87 patients (age at time of event 30 to 42 years; time after chemotherapy, 9-16 years): two patients with myocardial infarction and three patients with angina pectoris with proven myocardial ischemia. An increased observed-to-expected ratio of 7.1 (95% confidence interval, 1.9-18.3) for coronary artery disease, as compared with the general male Dutch population, was found. In addition, one patient experienced a cerebrovascular accident. Exercise-ECG did not reveal cases of subclinical coronary artery disease. Echocardiography showed normal systolic left ventricular function in most patients, but diastolic left ventricular function was disturbed in 33% of the patients. Of 62 chemotherapy patients, 79% had hypercholesterolemia, 39% had hypertension, 25% still experienced Raynaud's phenomenon, and 22 % had microalbuminuria. Compared with patients with stage I disease, the chemotherapy patients had higher blood pressure, higher total cholesterol- and triglyceride levels and were more insulin resistant.

Conclusion. In long-term survivors of metastatic testicular cancer we observed a significantly increased risk for occurrence of cardiac events accompanied by a persisting unfavorable cardiovascular risk-profile. Accurate follow-up, focused on cardiovascular complications and aimed at intervention in these young cancer-survivors seems to be important.

INTRODUCTION

Twenty years after the introduction of cisplatin containing chemotherapy for metastatic testicular cancer, this treatment remains one of the few with a high curative potential in disseminated cancer. The success of this therapy has resulted in an increasing number of patients who have been cured of testicular cancer. Because life expectancy of this group of young men is presumed to be normal once they have achieved a durable complete remission, the impact of long-term effects of the cytostatic treatment is becoming increasingly relevant. Attention has therefore been drawn to late sequelae of the chemotherapy such as nephrotoxicity and neurotoxicity.^{69,78,304} The described cardiovascular complications associated with cisplatin chemotherapy is a cause of increased concern about the ultimate long-term prognosis of these patients.^{10,40} We previously reported on the unfavorable cardiovascular risk profile that testicular cancer

survivors develop several years after chemotherapy.¹⁵ This unfavorable profile, which includes hypertension, overweight and an elevated serum level of total cholesterol in combination with a decreased high-density lipoprotein (HDL) cholesterol level, has also been described by others.^{14,16,73} The impact of cardiovascular risk factors in these young patients may be aggravated by the chemotherapy-associated vascular abnormalities such as Raynaud's phenomenon.^{74,75,79} However, because of a relatively limited follow-up, the clinical impact of these cardiovascular risk factors on health status remains unclear.

The aim of the present study is to evaluate the incidence of cardiovascular events, the presence of subclinical cardiovascular damage and the presence of other related late side effects of cisplatin combination chemotherapy administered to patients with disseminated testicular cancer 10 or more years ago.

PATIENTS AND METHODS

In October 1997 the charts of all 208 patients treated prior to October 1987 with cisplatin containing chemotherapy for disseminated testicular cancer at the University Hospital Groningen, the Netherlands, were reviewed for the occurrence of cardiovascular events. Patients who entered onto this analysis had to meet the following additional criteria: age 50 years or younger in October 1997; no evidence of testicular cancer for at least three years after initial treatment; a documented follow-up of at least 10 years; and no treatment with radiotherapy. A total of 87 patients met these criteria. The median age of these patients in October 1997 was 41 years (range, 30-50 years); the median follow-up duration was 14 years (range, 10-20 years). A cardiac event was defined as a fatal or non-fatal myocardial infarction or angina pectoris with proven myocardial ischemia. By the person-years approach, the ratio was calculated of the observed (O) number and the expected (E) number of cases with angina pectoris with proven myocardial ischemia plus cases with non-fatal myocardial infarction, using age- and sex specific incidence rates data from two Dutch registration projects.³⁰⁵ Data from these two registration projects originated from a total of 46 general practitioner practices and included data of 92633 men in the age between 15 and 50 years. Confidence limits for the ratio of the O and E numbers of patients were determined with the assumption of a Poisson distribution for O numbers. The person-years at risk were counted from the end of chemotherapy treatment through the last follow-up date. Person-years at risk were counted in patients who experienced either a cardiovascular event or a late tumor relapse before or on the date of diagnosis of the cardiovascular event or the date of tumor relapse.

Follow-up investigation

Of the 87 patients mentioned above, 77 were invited for prospective follow-up investigations with emphasis on actual cardiovascular disease and cardiovascular risk factors. Ten patients were not invited for the following reasons: one (patient A in table 2) died at the age of 39 years as a result of an acute myocardial infarction, six experienced late tumor relapse and three were lost to follow-up (10-11 years after chemotherapy). Of the 77 patients, 62 (81%) agreed to participate in additional follow-up investigations. The

median age of these 62 patients at the time of chemotherapy treatment was 27 years (range, 17-36 years). The median age at the time of follow-up investigation was 42 years (range, 30-50 years) with a median follow-up duration of 14 years (range, 10-20 years). Administered chemotherapy regimens are listed in table 1. To analyze the possible influence of the cumulative cisplatin dose on late sequelae, patients were divided in two groups.¹⁴ One group was composed of those patients who received ≤ 400 mg/m² (n=42). The other group consisted of those who received > 400 mg/m² cisplatin (n=20). Furthermore the cardiovascular risk profile of the 62 chemotherapy patients was compared with the cardiovascular factor profile of patients enrolled onto a surveillance program at our institution for stage I testicular cancer. Of 61 available stage I patients with comparable age and follow-up duration 40 (66%) agreed to participate in this assessment. These latter patients were disease-free after orchidectomy only and received no chemotherapy or radiotherapy. The median age of these 40 patients at the time of orchidectomy was 26 years (range, 18-38 years). At the time of cardiovascular risk factor assessment the median follow-up duration after orchidectomy was 14 years (range, 10-20) and the median age was 41 years (range, 29-50 years).

Table 1. Chemotherapy regimens of the 62 patients that participated in the follow-up investigation

Chemotherapy regimen*	No. of patients	(%)
PVB ⁶⁹	9	15
PVB + maintenance PV for one year ⁶⁹	20	32
BEP ³⁰⁶	16	26
PVB/BEP ³⁰⁶	11	18
EP ¹⁹⁵	4	6
EIP ³⁰⁷	2	3

* P = Cisplatin ; V = Vinblastine ; B = Bleomycin; E = Etoposide; I = Ifosfamide

Measurements

A detailed medical history was obtained on the day of the follow-up investigation with the help of a standardized questionnaire. This questionnaire included questions related to actual and previous cardiac function and to the presence of complaints known to be related to chemotherapy, especially Raynaud's phenomenon. Concerning Raynaud's phenomenon patients were asked the following questions: compared with before treatment, do/did you experience white, painful fingers or toes during emotion, while

touching cold objects, or during cold weather, followed by a burning feeling and redness of the acra? How did these symptoms develop over time? How would you best describe the severity, do/did they affect your daily life? Patients who regularly did experience or had experienced these symptoms were considered having Raynaud's phenomenon. Furthermore, the family history (first-degree kinship) for cardiovascular disease as well as the use of medication and tobacco was documented. A full physical examination was performed. Blood pressure was measured at both arms in a sitting position after a rest-period of 10 min with a standard mercury sphygmomanometer. Hypertension was defined as systolic pressure of more than 150 mmHg or a diastolic pressure of more than 95 mmHg or by the prescribed use of anti-hypertensive medication. The body mass index (BMI), defined as body weight (kg) divided by height² (m²) was used as a measure of corpulence. A patient with a BMI of more than 27.8 kg/m² was considered overweight.³⁰⁸

Chemotherapy treated patients underwent an exercise ECG by a bicycle ergometer according to a standard protocol³⁰⁹ to evaluate the existence of silent myocardial ischemia. Patients with abnormal test results underwent further evaluation by standard stress and rest myocardial perfusion scintigraphy using ^{99m}Tc-sestamibi.³¹⁰ Echocardiograms were also performed using a Vingmed CFM 800 (Sonotron, Horten, Norway) equipped with a 3.25 MHz transducer. Two-dimensional images were obtained in the parasternal long-axis and short-axis views and the apical four-chamber and two-chamber views. Transmitral Doppler flow velocity patterns were recorded from the apical four-chamber view.²⁶³ The wall motion score index (WMSI) was used as an overall measure of systolic left ventricular function.³¹¹ A WMSI > 1.5 was considered to indicate significant systolic dysfunction. The early peak flow velocity / atrial peak flow velocity (E/A) ratio was used to assess diastolic left ventricular function to test for early left ventricular dysfunction. An E/A ratio < 1.0 was considered to indicate diastolic dysfunction.³⁰⁹ The left ventricle mass (LVM) was calculated by the formula of Devereux and was indexed for body surface area.²⁶³ A LVM-index (LVMI) of > 125 g/m² body surface area was considered to indicate left ventricular hypertrophy (LVH).³¹²

Blood samples collected after an overnight fast were analyzed for serum levels of total cholesterol, triglycerides, magnesium (Mg) and creatinine by the Vitros 750c analyzer (Ortho Clinical Diagnostics, Beerse, Belgium). The HDL cholesterol and low-density lipoprotein (LDL) cholesterol levels were determined as previously described.^{313,314} Cut-off points for lipid levels were used as recommended by the American National Education Program.³¹⁵ Hypercholesterolemia was defined as an elevated fasting total serum cholesterol level (> 5.2 mmol/l) or the use of cholesterol-lowering medication.

Because Raynaud's phenomenon frequently occurs in these chemotherapy treated patients, they were evaluated for endothelium dysfunction as tested by the urinary albumin excretion in 24-hour urine collections using radioimmunoassay (RIA) (EURO/Diagnostics Products Corporation, Lanberis, UK). Microalbuminuria was defined by an albumin excretion > 20 µg/min.^{61,316} Creatinine excretion in 24-hour urine collection was determined to estimate renal function. Because the sex hormonal balance is often disturbed^{15,94} levels of serum testosterone, estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were determined using RIAs. Thyroid-stimulating

hormone and thyroxin (T4) levels were measured with RIAs to exclude hypothyroidism as the cause of hypercholesterolemia. Fasting insulin and glucose levels were studied to investigate whether these patients who were inclined to being overweight developed insulin resistance.¹⁵ Insulin was measured by RIA, and glucose was measured using the glucose oxidase method by using the ECA 180 analyzer (Medingen, Dresden, Germany). As a measure for insulin resistance, the insulin-to-glucose ratio (IGR) was calculated by dividing fasting serum insulin (pmol) by fasting serum glucose (mmol). An IGR of more than 22 was considered to indicate insulin resistance.³¹⁷

Statistical analysis

The 95% confidence interval (CI) of the O/E ratio was obtained using a Poisson distribution of O numbers.³¹⁸ Mean or median values of different groups and subgroups were compared with an unpaired Student's t-test or Mann-Whitney *U* test when indicated. Categorical variables were compared using the Chi-square test. Pearson's correlation coefficient and the Spearman rank-sum tests were applied when indicated. Multiple-linear regression analysis was performed to evaluate the effect of different cumulative cytostatic dosages or cytostatics administration on the cardiovascular parameters with adjustment for potential confounding factors. Unless otherwise stated, data are expressed as mean $\pm$ SD. All tests were two-sided; *P* values less than .05 were considered to indicate significance.

RESULTS

Cardiovascular events

Cardiac events occurred during follow-up after completion of chemotherapy in five (6%) of the 87 evaluated patients. These events occurred in two patients who had experienced myocardial infarction (being fatal in one), and in three patients who developed angina pectoris with proven ischemia. None of these five patients did have any cardiac problems before the start of chemotherapy. Details of the patients with cardiac events are listed in table 2. A comparison with the general Dutch male population revealed an O/E ratio of 7.1 (95% CI: 1.9-18.3) for cases of non-fatal myocardial infarction plus cases of angina pectoris with proven ischemia. The age range of the patients at the time of the event was from 30 to 42 years. All events occurred between 9 and 16 years after chemotherapy. All of the affected patients had developed hypertension in the course of time after chemotherapy, and three of these patients had additional cardiovascular risk factors. The patients with angina pectoris underwent a revascularization procedure or are currently being treated with antianginal medication. In addition, one patient experienced a cerebrovascular accident at the age of 41 years, 11 years after chemotherapy (PVB). He had hypertension and hypertriglycerideemia.

Table 2. Characteristics of patients with cardiac events following chemotherapy for disseminated testicular cancer

Patient	Cardiovascular disease	Age at time of event (yrs.)	Chemother. regimen	Time after chemother. (yrs.)	Additional cardiovascular risk factors
A	Fatal MI	39	PVB/BEP	12	hypertension
B	Acute MI	42	PVB+	16	hypertension, smoker, positive family history
C	AP*	36	PVB+	16	hypertension, hypercholesterolemia, overweight
D	AP*, LVH	39	PVB/BEP	10	hypertension, hypercholesterolemia
E	AP**	30	EIP	9	hypertension

MI = myocardial infarction; AP = angina pectoris; LVH = left ventricular hypertrophy; PVB = cisplatin, vinblastine, bleomycin; BEP = bleomycin, etoposide, cisplatin; EIP = Etoposide, ifosfamide, cisplatin;

* disease documented by coronary angiography ** ischemia documented by ^{99m}Tc -Sestamibi-scan and PET-scan

Follow-up investigation

Cardiac damage

Fifty-five asymptomatic chemotherapy patients underwent an exercise ECG to test for silent myocardial ischemia. The test showed significant ST depression during exercise in six of the 55 patients (11%). However, additional investigation by a ^{99m}Tc -Sestamibi-scan in these six patients could not confirm signs of ischemia. Reliable echocardiographic images could be obtained in 58 of the 62 chemotherapy patients, including the four patients with a previous cardiac event. Systolic left ventricular function was disturbed ($\text{WMSI} > 1.5$) in two patients, including patient D (table 2). One patient (age at follow-up: 30 years) had echocardiographic evidence of a previous myocardial infarction without a history of clinical symptoms. He was a former smoker and had hypercholesterolemia. Evidence of disturbed diastolic left ventricular function was found in 19 (33%) patients. The mean E/A ratio was 1.1 ± 0.29 (range, 0.44-1.97). The E/A ratio was significantly lower for patients who received a cumulative dose $> 400 \text{ mg/m}^2$ compared to those who received $\leq 400 \text{ mg/m}^2$ cisplatin, 0.99 vs. 1.15 respectively ($p=0.04$). Mean systolic blood pressure was higher in the patients with an E/A ratio of less than 1.0 compared to those with a normal E/A ratio, $151 \pm 25 \text{ mmHg}$ vs. $136 \pm 19 \text{ mmHg}$ respectively ($p=0.02$). Mean diastolic pressure did not significantly differ. Of the 19 patients with an E/A ratio < 1.0 , 10 (53%) had normal blood pressure. No influence of cumulative cytostatics dose or administration could be detected on the

E/A ratio after adjusting for age, heart rate and systolic blood pressure. Of the 55 chemotherapy patients in whom the LVM index could be calculated, six (11%) had LVH. All six patients had hypertension.

Cardiovascular risk factors

Sixty (97%) of the 62 chemotherapy patients had each one or more cardiovascular risk factors (hypertension, hypercholesterolemia, a smoking history or a positive family history for cardiovascular events). Twenty-four (39%) of the 62 chemotherapy patients had hypertension. The blood pressure before the start of chemotherapy was normal in all of these patients. Of the 24 patients with elevated blood pressure, 11 used anti-hypertensive medication. The median time between chemotherapy treatment and the diagnosis of hypertension was 7.5 years (range, 2-17 years), at a median age of 35 years (range, 28-42 years). Chemotherapy patients with hypertension had a higher left ventricular posterior wall thickness compared to normotensive patients, 9.5 ± 1.4 mm vs. 8.7 ± 1.4 mm ($p=0.02$). Compared with the stage I patient group, hypertension was found more frequently in the chemotherapy group (13% vs 39%; $p=0.003$). Both the mean systolic and diastolic blood pressure in the chemotherapy group were significantly higher compared with the stage I group (table 3). Forty-nine (79%) of the 62 chemotherapy patients had hypercholesterolemia, including seven patients who used cholesterol-lowering medication. The comparisons of lipid levels of the chemotherapy group with those of the group with stage I disease are summarized in table 3. The mean total cholesterol, the mean total cholesterol/HDL cholesterol ratio and mean triglycerides were significantly higher in the patients who received chemotherapy compared with those of the patients who were treated with orchidectomy only. The differences in lipid levels and blood pressure between both groups could not be attributed to difference in age because the mean age was the same in the two groups.

Twenty-two (36%) of the 62 chemotherapy patients experienced symptoms of Raynaud's phenomenon during follow-up, but in seven patients (11%) Raynaud's phenomenon had subsided. Serum Mg level in patients with Raynaud's phenomenon was not significantly lower compared to patients without Raynaud's phenomenon 0.82 ± 0.08 mmol/L ν 0.88 ± 0.11 mmol/L ($p=0.06$). Eleven (22%) of 51 evaluated chemotherapy patients had microalbuminuria with a median albumin excretion of 80 μ g/min (range 22.2-718.1). This microalbuminuria is considered to be a sign of endothelial damage. Chemotherapy treated patients with microalbuminuria had a higher mean systolic and diastolic blood pressure when compared to patients without increased albumin excretion (Systolic blood pressure 157 ± 32 mmHg ν 136 ± 17 mmHg; $P=.05$; Diastolic blood pressure 100 ± 11 mmHg ν 88 ± 11 mmHg; $P=.004$, respectively). The mean creatinine clearance of all 62 chemotherapy patients was 111 ± 24 mL/min. The creatinine clearance was decreased (< 100 mL/min) in 19 patients (31%). The mean serum Mg level was 0.85 ± 0.08 mmol/L and was below normal (< 0.74 mmol/L) in five patients. The serum Mg level correlated inversely with the cumulative cisplatin dose ($r=-.318$; $P=.02$).

Thirteen of the chemotherapy patients (21%) were overweight compared with 11 of the stage I patients (28%). The mean BMIs of the chemotherapy group and the group with stage I disease did not differ significantly (25.8 ± 3.6 kg/m² ν 25.7 ± 3.5 kg/m²; P

Table 3. Cardiovascular risk factors in long-term survivors of testicular cancer after chemotherapy (n=62) or orchidectomy only (n=40)

Risk factor	Normal value	Chemotherapy group (n=62)			Stage I group (n=40)			P-value ^{*1}
		Mean ± SD	Range	Number (%) of patients with abnormal result	Mean ± SD	Range	Number (%) of patients with abnormal result	
<i>Dyslipidaemia</i>				49 (79) ^{*3}			23 (58) ^{*3}	0.02
Total Cholesterol	< 5.2 mmol/L	6.2±1.2 ^{*2}	4.3-9.7	49 (79) ^{*3}	5.6±1.2 ^{*2}	3.5-9.8	23 (58) ^{*3}	0.03
HDL-Cholesterol	> 0.9 mmol/L	1.0±0.3 ^{*2}	0.3-1.7	19 (35) ^{*3}	1.1±0.3 ^{*2}	0.7-2.1	10 (25) ^{*3}	0.16
LDL-Cholesterol	< 3.4 mmol/L	4.3±1.1 ^{*2}	1.1-6.5	49 (79) ^{*3}	3.9±1.1 ^{*2}	2.2-8.0	24 (62) ^{*3}	0.18
Total Chol./HDL-chol.-ratio	< 5.0	6.8±3.0 ^{*2}	3.2-22	44 (71) ^{*3}	5.5±1.9 ^{*2}	2.7-11	21 (53) ^{*3}	0.02
Triglycerides	<2.3 mmol/L	2.2±2.3 ^{*2}	0.8-14	14 (25) ^{*3}	1.4±0.8 ^{*2}	0.2-4.1	4 (10) ^{*3}	0.02
<i>Hypertension</i>				24 (39) ^{*4}			5 (13) ^{*4}	0.003
Diastolic pressure	≤ 95 mmHg	90±12 ^{*5}	65-140	24 (39) ^{*6}	82±11 ^{*5}	65-105	3 (8) ^{*6}	0.003
Systolic pressure	≤150 mmHg	137±18 ^{*5}	110-190	22 (35) ^{*6}	123±14 ^{*5}	95-153	1 (3) ^{*6}	<0.001
<i>Family history positive for cardiovascular events</i>				23 (37)			10 (25)	0.20
<i>Smoking</i>								
Former Smoker				20 (32)			10 (25)	0.43
Current Smoker				19 (31)			9 (23)	0.37

^{*1}Chemotherapy group v group with stage I disease; ^{*2}Patients using cholesterol-lowering medication not included; ^{*3} Including patients using cholesterol lowering medication; ^{*4}Definition: diastolic pressure > 95 mmHg / systolic pressure > 150 mmHg or the use of antihypertensive medication; ^{*5} Patients using antihypertensive medication not included; ^{*6} Including patients using antihypertensive medication

=.86). The comparison of fasting hormone levels of both the 62 chemotherapy treated patients and the 40 patients with stage I disease at follow-up investigation is summarized in table 4. Fasting glucose levels for all but one patient were normal (< 7.8 mmol/L) and did not differ between the two groups. Both the median insulin level and the median IGR as a measure for insulin resistance, however, were significantly higher in the chemotherapy group when compared with the group with stage I disease (table 4). The median testosterone level in the chemotherapy group was significantly lower when compared with the stage I group (table 4). After adjusting for BMI the IGR correlated inversely with testosterone level in the chemotherapy group ($r = -0.32$; $P = .015$) but not in the group with stage I disease ($r = 0.18$; $P = .27$). In the chemotherapy group a decreased testosterone level (< 11 nmol/L) was found in six of the patients (10%), while the FSH level was elevated in 42 (68%) and the LH level was above normal in 12 (19%). However, the median FSH and LH levels did not differ significantly between the chemotherapy and stage I group. This indicates that subclinical Leydig-cell dysfunction still exists in both patient groups 10 to 20 years after treatment, although more pronounced in the chemotherapy group. All patients had normal thyroid-stimulating hormone and T4 levels, though the median T4 level was significantly lower in the chemotherapy patients when compared with that of the patients with stage I disease.

DISCUSSION

With the increasing number of long-term survivors of metastatic testicular cancer treated with cisplatin containing chemotherapy, the impact of an increased incidence of cardiovascular risk factors has become a matter of great concern. The results of our current study, which involved a follow-up of 10 to 20 years after chemotherapy feed this concern. In the studied cohort of 87 patients, all of whom were 30 to 50 years of age at the time of follow-up, five (6%) experienced major cardiac events 9 to 16 years after treatment. The high O/E ratio (7.1 (95% CI: 1.9-18.3)) for non-fatal myocardial infarction plus angina pectoris with proven myocardial ischemia in this cohort strongly suggests that, in long-term survivors of metastatic testicular cancer cured with cisplatin containing chemotherapy, the cardiac event rate might be elevated compared with that of the general population.

Previous follow-up studies of survivors of metastatic testicular cancer have reported somewhat lower prevalences of major cardiac events, ranging from 0% to 3%.^{13-15,37,40,68,69,319-327} The higher prevalence of cardiac events (6%) that we found may be explained by the fact that the median follow-up duration after chemotherapy in our study was 14 years, versus 1 to 7 years in previous studies. All cardiac events in our study, occurred long after chemotherapy (9 to 16 years), which illustrates the importance of a long follow-up duration to reveal the full impact of the chemotherapy on the cardiovascular status of these cancer survivors. In none of the earlier reports were comparisons made with incidence rates of cardiac events of the local normal population. Therefore, a comparison with our finding is difficult. Whether this observation of a greater-than-expected number of cardiac events is the result of the administered chemotherapy or the natural history of testicular cancer is not clear. Because the total

Table 4. Hormone levels in long-term survivors of testicular cancer after chemotherapy (n=62) or orchidectomy only (n=40)

Variable	Normal value	Chemotherapy group (n=62)			Stage I group (n=40)			P-value
		Median	Range	Number (%) of patients with abnormal result	Median	Range	Number (%) of patients with abnormal result	
Glucose	<7.8 mmol/L	4.8	2.2-10	1(2)	4.7	4.0-7.2	0(0)	0.75
Insulin	< 10 mU/L	10.5	2.9-48	34 (55)	7.7	3.3-24	11(28)	0.013
IGR ^{*2}	< 22	16.5	4.5-83	13 (22)	11.7	5.6-38	4(10)	0.015
Testosterone	> 11 nmol/L	16.1	5.9-25	6(10)	18	10-31	1(3)	0.023
Estradiol	> 0.22 nmol/L	0.14	0.06-0.24	3(5)	0.16	0.04-	5(13)	0.37
LH	< 8 U/L	4.1	0.3-19	12(19)	3.5	1.1-14	6(15)	0.85
FSH	< 7 U/L	10.6	2.6-47	42(68)	8.8	2.5-45	24(62)	0.4
TSH	< 5 mU/L	1.1	0.3-3.5	0(0)	0.9	0.4-2.9	0(0)	0.18
FT4	< 9pmol/L	12.8	6.1-20	3(5)	14.3	9.3-21	0(0)	<0.001

*1 Median hormone levels of both groups were tested using the Mann-Whitney U-test.

*2 Insulin-to-glucose ratio

number of patients and events in this study is small and the interval between treatment and occurrence of cardiac events is long, possible confounding factors such as genetic predisposition for cardiovascular disease and environmental factors may have influenced the current observation.

Because of the increased cardiac event rate that we found in the chemotherapy treated patients, additional analysis of subclinical cardiac disease was performed. Exercise-ECG testing and when indicated ^{99m}Tc -sestamibi scintigraphy did not reveal additional cases of subclinical coronary artery disease. Echocardiographic evaluation, however, showed evidence of a previous silent myocardial infarction in one additional patient. Furthermore, although the systolic left ventricular function was normal in most patients, 33% of the chemotherapy patients had abnormal diastolic function of the left ventricle which suggested early-stage cardiac dysfunction. Left ventricular diastolic dysfunction has been observed in several conditions, including hypertension, coronary artery disease and diabetic mellitus.^{253,328,329} The left ventricular diastolic dysfunction in the patients studied correlated with age and systolic blood pressure, although approximately one half of the patients with disturbed left ventricular diastolic function had normal blood pressure. The chemotherapy administered, especially the cumulative dosage of cisplatin, was not determined to be an important causal factor, possibly because it was only given in small variations in administered dosages. If the observed diastolic dysfunction reflects the presence of small vessel abnormalities in the myocardium, then this might affect the development of future cardiac events. The same can be stated for LVH, which is known to increase the risk of cardiac events.^{330,331} In our patients, LVH is probably the result of long-standing hypertension.

It remains unresolved whether the high number of cardiac events and the observed echocardiographic signs of cardiac damage represent a direct toxic effect of the administered chemotherapy or a result of the increased incidence of cardiovascular risk factors or other secondary changes that occurred in this patient group. Although the cause-effect relationship between this type of chemotherapy and Raynaud's phenomenon is well established,⁹ the role of small vessel abnormalities in the pathogenesis of major cardiovascular events in these patients is not clear. Raynaud's phenomenon still persists up to 20 years after chemotherapy in 25% of our patients. Furthermore, we found that 22% of the patients who received chemotherapy had microalbuminuria, which suggests endothelial damage. Because microalbuminuria has been identified as an independent risk factor for cardiovascular disease in large trials, this expression of small vessel disease might contribute to the high cardiac event rate.^{98,332} Clustering of risk factors was found in our chemotherapy patients with microalbuminuria. Patients with microalbuminuria showed elevated blood pressure, which suggests that endothelial damage might be a pathogenic factor in the development of hypertension.⁶¹

In 1992 we reported on the increased occurrence of cardiovascular risk factors in patients cured with chemotherapy from disseminated testicular cancer.¹⁵ These findings have been confirmed by other groups.^{14,16,73} The current study demonstrates that this increase in risk factors persists up to 20 years after chemotherapy and may have a substantial influence on the development of cardiovascular events found in the present study. The reason these patients develop this unfavorable cardiovascular risk profile is

still unknown. Comparing the cardiovascular risk profile of the chemotherapy treated patients with stage I disease who did not receive chemotherapy revealed a significantly higher blood pressure, higher total cholesterol level, higher total cholesterol/HDL-cholesterol-ratio and higher triglyceride level in the chemotherapy group. Because both groups were comparable in terms of age and follow-up duration, these differences may be caused by the administered chemotherapy.

Besides the direct toxicity of chemotherapy, secondary metabolic and hormonal changes in patients treated with chemotherapy may play a role in the development of cardiovascular morbidity. Elevated LH and FSH levels were found in a large portion of patients in both the chemotherapy group and the group with stage I disease. This indicates persistent Leydig-cell dysfunction.^{15,91,92,94} However, in addition we found that testosterone levels were significantly lower in the chemotherapy treated patients, which suggests a more pronounced damage of gonadal function in these patients. Since the negative correlation between the marker of insulin resistance (IGR) and testosterone was demonstrated only in the chemotherapy treated patients this might be interpreted as a metabolic consequence of long term gonadal toxicity of the chemotherapy. The combination of overweight, microalbuminuria, hypertension, insulin resistance and dyslipidemia suggests the presence of a metabolic syndrome-X like state⁹⁹ in the patients who were cured with chemotherapy. This combination of factors might influence the high incidence of cardiovascular risk factors and increased cardiac event rate observed in patients treated with cisplatin containing chemotherapy.

Our results should be interpreted within the framework of a small cohort study. To determine with certainty why patients cured from disseminated testicular cancer can develop cardiovascular disease, case-control studies with healthy controls and with patients with testicular cancer patients have not received chemotherapy are needed.

This study illustrates the importance of accurate long-term follow-up of patients who have been cured of disseminated testicular cancer with cisplatin containing chemotherapy. The risk of tumor relapse decreases with increasing follow-up. The risk of a second malignancy, however, increases gradually.³³³ We found that the risk of developing a major cardiac event is higher than expected in these young patients. The development of cardiovascular disease might be a greater threat to these patients than developing a second malignancy. Although several pathogenic mechanisms such as endothelial damage, sex hormonal imbalance and insulin resistance may contribute to the development of cardiovascular problems in these patients, the exact mechanism is still unclear and needs to be unraveled so that rational intervention measures can be designed. Further guidelines need to be developed in the short term to prevent or treat cardiovascular risk factors in these patients with the ultimate goal of improving life expectancy.

Acknowledgements

We thank M. Schaapveld and R. Gijssen for assistance in analyzing a part of the data.

CHAPTER 5

Circulating plasma platinum more than 10 years after cisplatin treatment for testicular cancer

*J. A. Gietema¹, M. T. Meinardi¹, J. Messerschmidt³, T. Gelever², F. Al³, D.R.A. Uges²,
D. Th. Sleijfer¹*

Departments of Medical Oncology¹ and Hospital Pharmacy²,
University Hospital Groningen, The Netherlands;
Institute of Spectrochemistry and Applied Spectroscopy³,
Dortmund, Germany

The Lancet 2000; 355: 1075-6

We have shown in patients cured from metastatic testicular cancer that up to 20 years after administration of cisplatin-containing chemotherapy, circulating platinum is still detectable in plasma. This finding may influence the development of long-term, treatment-related side-effects.

More than two decades after the introduction of cisplatin-containing chemotherapy for metastatic testicular cancer, this treatment remains one of the few with a high curative potential in disseminated cancer. The success of this therapy has resulted in increased survival in patients who have had testicular cancer. Therefore, the long-term sequelae of cytostatic treatment, such as cardiovascular problems or secondary malignancies, is becoming increasingly relevant.^{15,333} The pathogenesis of the long-term sequelae of cisplatin combination chemotherapy in patients with testicular cancer has not yet been fully elucidated but it has been associated with prolonged retention of platinum in the body.

During a long-term follow-up investigation in 61 testicular-cancer patients cured with cisplatin combination chemotherapy more than 10 years previously, we measured plasma platinum concentrations using a sensitive assay. The median age of the patients at the time of chemotherapy was 27 years (range 17-36 years). The median age at the time of follow-up investigation was 42 years (30-50 years), with a median follow-up of 14 years (10-20 years). Patients were treated with four courses of cisplatin, bleomycin, and vinblastine or etoposide, every 3 weeks. 17 patients additionally received maintenance therapy with vinblastine and cisplatin for a maximum of 1 year. The total amount of administered cisplatin per patient ranged from 350 to 950 mg/m² (663-1846 mg). The 44 patients without maintenance chemotherapy received a mean cisplatin dose of 400 mg/m² (SD 14; range 350-450 mg/m² [663-987 mg]), and the 17 patients who also were treated with maintenance chemotherapy received a mean cisplatin dose of 801 mg/m² (SD 99, range 600-950 mg/m² [1191-1846]). Plasma platinum concentrations in these patients were compared with those of 20 control patients who were cured from stage I testicular cancer by orchidectomy without chemotherapy. The median age of the control patients at the time of orchidectomy was 26 years (18-38 years). The median age at follow-up was 42 years (30-50 years), with a median follow-up duration of 14 years after orchidectomy (10-20 years). Platinum concentrations were measured in masked plasma samples by a sensitive procedure during which high-pressure decomposition of samples is followed by an adsorptive voltammetric measurement.³³⁴ The limit of quantification of platinum was 6 pg/g plasma. Measurements were done in duplicate; the coefficient of variation and day-to-day variation were 6% and 5%, respectively.

The platinum concentrations in the plasma of the 61 patients 10-20 years after cisplatin administration were significantly higher than those of the 20 control patients (cisplatin group: mean platinum concentration 64·9 pg/g plasma [SD 24·5] vs control group: 18 patients with platinum concentrations below the limit of detection and two patients with platinum concentrations at the limit of detection; Mann-Whitney U test, $p < 0\cdot0001$; figure 1). In all chemotherapy patients, the plasma platinum concen-

trations were above the limit of quantification, indicating that up to 20 years after treatment, there is still retention of platinum in the plasma. The concentration-time curve shows a gradual decline in plasma platinum concentration (figure 2). For the 61 patients there was a significant correlation between plasma platinum concentrations and follow-up duration ($r=-0.365$; $p=0.004$). When adjusting for follow-up duration after chemotherapy, the plasma platinum concentrations correlated with administered cisplatin dose ($r=0.61$; $p<0.001$), as well as with creatinine clearance before treatment ($r=-0.34$; $p=0.009$) and at follow-up ($r=-0.40$; $p=0.002$). Although we did not measure urinary platinum content, one might assume that plasma clearance is the result of urinary platinum excretion since the kidney is the main route of cisplatin elimination.²⁶ From the patients treated with 400 mg/m² cisplatin, a half-life of 4.6 years was estimated. This very long half-life suggests that part of the retained platinum is recirculating in the plasma between potential body stores such as the liver, bone, and muscle.²⁵

These data indicate that patients with disseminated testicular cancer, of whom 80-90% can be cured with standard 400 mg/m² cisplatin-containing chemotherapy, will be exposed to platinum for at least 20 years. Whether chronically raised platinum concentrations in these ex-patients will have an influence on the development of late toxicity and secondary malignancies is not known. Further study is needed to define the role of platinum retention in the late sequelae of cancer treatment, and to find possible ways to circumvent these effects.

Figure 1. Plasma platinum concentrations of 61 cured testicular cancer patients 10-20 years after cisplatin combination chemotherapy and 20 cured testicular cancer patients 10-20 years after orchidectomy
Horizontal line=limit of quantification of platinum (6 pg/g)

Figure 2. Concentration-time plot of plasma platinum concentrations of 61 cured testicular cancer patients 10-20 years after cisplatin combination chemotherapy

CHAPTER 6

Magnesium and phosphorus

J. A. Gietema¹, M.T. Meinardi¹, W.T.A. van der Graaf¹, D.R.A. Uges², D.Th. Sleijfer¹

Departments of Medical Oncology¹ and Hospital Pharmacy²,
University Hospital Groningen, The Netherlands

The Lancet 1998; 352: 1476

Sir--José R Weisinger and Ezequiel Bellorín-Font³³⁵ claim that after a period with a negative magnesium balance an equilibration between serum magnesium and body stores occurs within several weeks. Our data suggest that such an equilibration might not occur for up to 20 years after cisplatin-induced renal loss of magnesium.

Cisplatin causes proximal-tubule damage that results in renal magnesium wasting.³⁰ How long this magnesium depletion persists after treatment is not known. We treated 50 patients with disseminated testicular cancer between 1978 and 1988. The patients received four courses of cisplatin-containing chemotherapy and were followed up for 20 years after treatment to assess the long-term effects of therapy. At a median follow-up of 14 years (range 10-20), magnesium status was examined. Since serum magnesium is a poor predictor of magnesium body stores, red-blood cell magnesium concentration was measured by an indirect method.³³⁶ Serum and red-blood cell magnesium concentrations were compared with a control group of 17 patients with testicular cancer not treated with chemotherapy. None of the patients used magnesium suppletion, diuretics, or known drugs that induce magnesium loss.

We found that the mean serum concentration of magnesium of both groups did not differ significantly (0.85 [SD 0.08] vs 0.84 [0.07] mmol/L; normal value 0.75 - 0.95 mmol/L). Five patients in the chemotherapy group had a serum concentration of magnesium below 0.75 mmol/L, whereas one of the control patients had a concentration below 0.75 mmol/L. However, the red-blood cell magnesium content in the group of patients 14 years after cisplatin chemotherapy was significantly lower than the control group (2.12 [0.19] vs 2.75 [0.35] mmol/L, $p=0.004$). 24-h urinary excretion of magnesium in the chemotherapy group showed large variation.

Since the urinary excretion of magnesium in none of the patients was below 2 mmol/24 h, a non-renal cause of magnesium depletion is unlikely.³³⁷ On the other hand, only six patients had a renal magnesium loss of more than the upper limit of 8 mmol/24 h. No correlation could be detected between 24 h urinary magnesium excretion and serum or red-blood cell magnesium concentration. These data suggest that, although patients who were treated 10-20 years ago with cisplatin-containing chemotherapy have a normal serum concentration of magnesium, there are signs that they are still magnesium deficient. Our data indicate that a magnesium equilibrium is not reached in cases up to 20 years after chemotherapy.

We previously reported on the increased cardiovascular risk profile found during long-term follow-up in patients with testicular cancer cured with chemotherapy.¹⁵ Whether the coincidence in these patients of magnesium depletion and hypercholesterolemia with increased bodyweight is the result of metabolic alterations secondary to deficiency of magnesium, as suggested by Weisinger and Bellorín-Font, is not known.

CHAPTER 7

Early detection of anthracycline induced cardiotoxicity in asymptomatic patients with normal left ventricular systolic function: autonomic versus echocardiographic variables

*G. Tjeerdsma¹, M.T. Meinardi², W.T.A. van der Graaf², M. P. van den Berg¹,
N. H. Mulder², H.J.G.M. Crijns¹, E.G.E. de Vries², D.J. van Veldhuisen¹*

Departments of Cardiology¹ and Medical Oncology²,
University Hospital Groningen, Groningen, The Netherlands

Heart 1999; 81:419-423

SUMMARY

Objective. To investigate left ventricular dysfunction in patients who had been treated with anthracycline based chemotherapy.

Methods. Autonomic function was compared with left ventricular diastolic function in 20 asymptomatic women with normal systolic function (left ventricular ejection fraction (LVEF) > 0.50) treated for breast cancer with high dose anthracycline based chemotherapy, and 20 age matched healthy controls. Left ventricular diastolic function was assessed echocardiographically by measuring the early peak flow velocity to atrial peak flow velocity ratio, isovolumic relaxation time, and deceleration time. Heart rate variability analysis was assessed for time domain and frequency domain parameters.

Results. The mean (SD) age of the patients was 45 (7) years and the mean LVEF was 0.59 (0.06). The time interval after the end of chemotherapy was 29 (27) months. One or more diastolic variables were abnormal in 50% of the patients. Heart rate variability was abnormal in 85% of patients. Mean values of both time domain and frequency domain parameters were decreased ($p < 0.05$), in particular the parasympathetic indices.

Conclusions. Autonomic impairment occurs in a large proportion of asymptomatic patients with normal systolic left ventricular function after high dose anthracycline based chemotherapy. In particular, heart rate variability analysis may be a sensitive tool to identify the first signs of cardiotoxicity in these patients.

INTRODUCTION

Anthracycline induced cardiotoxicity, which may ultimately lead to congestive heart failure, is a well known problem in the treatment of cancer and limits the therapeutic use of this group of effective antineoplastic agents.^{2,208} However, from several studies it is apparent that the inclusion of anthracyclines in chemotherapeutic regimens lessens the risk of disease progression and mortality.^{338,339} As a result, anthracyclines have been used increasingly in recent years, not only in patients with metastatic breast cancer but also in the adjuvant setting.^{340,341} Thus it is likely that cardiotoxicity will occur more often in the future. To optimize anthracycline treatment in the individual patient and to prevent cardiac injury, early detection of cardiotoxicity is needed. In many cancer treatment protocols, cardiotoxicity is assessed by measuring left ventricular ejection fraction (LVEF) by radionuclide ventriculography. However, LVEF is known to be a rather insensitive measure of early cardiac damage²¹ and several studies suggest that in anthracycline induced cardiotoxicity diastolic changes precede systolic dysfunction,^{22,255} as in other forms of heart disease.^{252,253} Thus the assessment of diastolic function may be a better method of early detection of cardiotoxicity.

Another method of detecting cardiotoxicity at an early stage may be the assessment of autonomic function. Although there has been increasing recognition in recent years of the role of autonomic imbalance in the pathogenesis of heart failure,^{342,343} up to now its value in anthracycline induced cardiotoxicity has hardly been studied. Autonomic changes have been shown to arise at an early stage of left ven-

tricular dysfunction and to increase with the severity of the disease.^{274,343,344} Heart rate variability analysis is a commonly used, reliable, non-invasive tool for assessing autonomic function.³⁴⁵

In this study we analyzed a group of asymptomatic female patients without left ventricular systolic dysfunction (LVEF > 0.50), who had been treated for locally advanced or metastatic breast cancer with high dose anthracycline based chemotherapy and, in most cases, with regional radiotherapy. We hypothesized that there would be echocardiographic evidence of diastolic dysfunction and impairment of heart rate variability parameters in this population.

METHODS

Patient selection

Asymptomatic (female) patients with locally advanced or metastatic breast cancer, who had been treated with anthracyclines (doxorubicin, epirubicin, mitoxantrone), were eligible for the study. These patients partly participated in a Dutch national randomized trial.³⁴⁶ None of these patients had a history of cardiac disorders, or was on cardiovascular drug treatment. In none of the patients was there evidence of diabetes mellitus or hypertension. All patients underwent cardiac evaluation, which included history and physical examination, radionuclide ventriculography to determine LVEF, echocardiography, and heart rate variability analysis. Only patients with an LVEF > 0.50 were included. Age matched healthy women served as a control group. The study was approved by the medical ethics committee of the University Hospital Groningen. All patients gave informed consent.

Chemotherapy regimen

Patients received one of the following three chemotherapy regimens (table 1):

(A) Four courses of 5-fluorouracil (500 mg/m²), epirubicin (90 mg/m²), and cyclophosphamide (500 mg/m²) for induction chemotherapy, followed by cyclophosphamide (6000 mg/m²), thiotepa (480 mg/m²), and carboplatin (1.6 g/m²) as ablative chemotherapy.³⁴⁶

(B) Six courses of 5-fluorouracil (1500 mg/m²), methotrexate (1500 mg/m²) alternating with doxorubicin (50 mg/m²), and vincristine (2 mg) for induction chemotherapy, followed by mitoxantrone (60 mg/m²) plus melphalan (180 mg/m²) or mitoxantrone (50 mg/m²) plus thiotepa (800 mg/m²) as ablative chemotherapy.³⁴⁷

(C) Three courses of cyclophosphamide (1500 mg/m²), epirubicin (80 mg/m²), 5-fluorouracil (1500 mg/m²), three courses of cyclophosphamide (1500 mg/m²), 5-fluorouracil (600 mg/m²) and methotrexate (1500 mg/m²), and two courses of high dose epirubicin (180 mg/m²) for induction chemotherapy, followed by thiotepa (800 mg/m²) and subsequently mitoxantrone (60 mg/m²) and melphalan (180 mg/m²) as ablative chemotherapy.

Regimen A contains intermediate dose anthracyclines, regimens B and C contain high dose anthracyclines (table 1).

Table 1. Patient characteristics of the study population.

Patient	Age (years)	LVEF	Time since chemother. (months)	Treatment		
				Induction chemother.	Ablative chemother.	Radiother. Chest side /Gy
1	30	0.70	4	C	TMM	L/50
2	51	0.54	38	B	MM	R/50
3	40	0.52	48	C	TMM	L/70
4	51	0.53	66	B	TM	L/50
5	47	0.59	6	C	TMM	-/-
6	43	0.73	8	A	CTC	R/50
7	55	0.60	20	A	CTC	L/50
8	40	0.57	28	A	CTC	L/50
9	54	0.60	13	A	CTC	R/50
10	51	0.64	30	A	CTC	R/50
11	51	0.60	19	A	CTC	L/50
12	48	0.65	8	A	CTC	R/50
13	50	0.65	17	A	CTC	R/32
14	47	0.63	34	A	CTC	L/50
15	34	0.62	21	A	CTC	R/50
16	49	0.65	1	A	CTC	-/-
17	49	0.61	108	B	MM	-/-
18	39	0.61	54	B	TM	R/50
19	43	0.60	2	A	CTC	L/50
20	46	0.52	54	B	MT	L/50
Mean (SD)	45 (7)	0.59 (0.06)	29 (27)			

A: chemotherapy regimen A; B: chemotherapy regimen B; C: chemotherapy regimen C;
 CTC: cyclophosphamide + thiotepa + carboplatin ; L: left side of the chest; LVEF: left ventricular ejection fraction; MM: mitoxantrone + melphalan ; R: right side of the chest;
 TM: thiotepa + mitoxantrone; TMM: thiotepa + mitoxantrone + melphalan.

Echocardiography

To assess diastolic function, echocardiography was performed using a Vingmed CFM 800 instrument (Sonotron, Horten, Norway), equipped with a 3.25 MHz transducer. Gain setting was optimized to a level just below background noise. Cross sectional images were obtained in left ventricular parasternal long axis and short axis views, and

apical four chamber and two chamber long axis views.³⁴⁸⁻³⁵⁰ Left ventricular end diastolic diameter (normal mean (SD) value 47 (4) mm) and posterior wall diastolic thickness (normal mean value 9 mm (range 6 to 11)) were measured as described by Weyman.³⁵¹ For the analysis of diastolic function, early peak flow velocity / atrial peak flow velocity (E/A ratio), deceleration time, and isovolumic relaxation time were measured.^{22,352} As described in previous papers, normal values are 1.9 (range 0.7 to 3.1), 179 ms (range 139 to 219), and 76 ms (range 54 to 98) for age matched healthy women, respectively.²⁶³

Heart Rate Variability

All patients underwent 24 hour Holter monitoring. The results were compared with healthy age matched women. The data were recorded using a Marquette 8000 Holter system (Marquette Electronics Inc, Milwaukee, Wisconsin, USA). Tapes with more than 15% noise overall were excluded, and heart rate variability was analyzed only when sinus rhythm was present. Time and frequency domain parameters were calculated as previously described in detail from our institution.^{274,353}

Briefly, time and frequency domain parameters were calculated from the time series of RR intervals and were computed over 288 segments of five minutes for a total of 24 hours recording. The following time domain parameters were measured: standard deviation of heart period (SDNN, ms), standard deviation of five minute mean values of NNs for each five minute interval (SDANN, ms), proportion of successive NN differences > 50 ms (PNN50, %), and root mean square successive difference of NN intervals (rMSSD, ms). Frequency domain analysis was performed by using discrete Fourier transformation. Low frequencies (LF, 0.04 to 0.15 Hz), high frequencies (HF 0.15 to 0.4 Hz), and total power (TP, 0.0033 to 0.4 Hz) were calculated.

Statistical analysis

All values are expressed as mean (SD). The two tailed independent non-parametric Mann-Whitney U test was applied to compare the outcome of autonomic variables in patients and age matched healthy controls. A p value < 0.05 was considered statistically significant.

RESULTS

Study population

Twenty two consecutive asymptomatic patients attending the outpatient clinic of the department of medical oncology for follow up were referred for the study. Two patients were found to have an LVEF of <0.50 (0.39 and 0.43, respectively) and these were excluded. The remaining 20 patients form the present study population. Their characteristics are summarized in table 1. Their mean age was 45 years (range 30 to 55), the mean (SD) LVEF was 0.59 (0.06), and the interval from discontinuation of anthracycline treatment to evaluation was 29 (27) months.

Treatment protocol

Twelve patients received regimen A, five patients regimen B, and three patients regimen C (table 1). Eight patients received mitoxantrone in regimens B and C. Seventeen of the 20 patients received regional radiotherapy, 32-70 Gy, after chemotherapy. In all cases irradiation was given with 4-6 MeV photons from a linear accelerator in fractions of 2 Gy to the breast or chest wall, the supraclavicular, axillary, and internal mammary lymph nodes. In nine patients the irradiation was given to the left side of the chest.

Echocardiography

The mean E/A ratio was 0.98 (0.05), range 0.6 to 1.4, and 10 of the 20 patients had an abnormal value (E/A ratio < 1.9). The mean deceleration time was 180 (13) ms, range 96 to 338; the result was abnormal in three patients and all three also had an abnormal E/A ratio. Mean isovolumic relaxation time was 74.0 (3.5) ms, range 48 to 88; the result was abnormal in only one patient who also had an abnormal E/A ratio. Therefore at least one of three diastolic variables was abnormal in 10 of the 20 patients, and 10 patients had no abnormalities. Left ventricular end diastolic diameter and posterior wall diastolic thickness were normal in all patients after treatment.

Heart rate variability

Eleven patients had no significant ventricular arrhythmia's. In three patients asymptomatic non-sustained ventricular tachycardia (maximum frequency 187 beats/min, maximum duration 1 second) were observed. Non-sustained supraventricular tachycardia (maximum frequency 107 beats/min, maximum duration 2 seconds) was detected in six patients. Of the time domain parameters, SDANN and SDNN were not significantly different between patients and healthy controls, $p < 0.90$ (fig 1).

In contrast, PNN50 and rMSSD were significantly lower in patients than in healthy controls (fig 2). For patients and healthy controls the mean PNN50 was 5.9 (8.9)% and 8.5 (1.5)%, respectively ($p < 0.05$). The mean rMSSD was 24.7 (13.2) ms in patients and 30.9 (2.0) ms in controls ($p < 0.05$).

Mean frequency domain parameters of heart rate variability were decreased for all variables; low frequency, high frequency, and total power spectrum were lower in patients than in controls (fig 3), $p < 0.01$. Mean HF was 8.7 (3.5) ms for patients ν 12.6 (0.9) ms for controls. Mean LF was 18.4 (6.6) ms for patients ν 23.7 (1.1) ms for controls. For the TP spectrum, the mean outcomes were 28.1 (8.7) ms and 35.6 (1.7) ms, respectively. Overall, 15% of the patients had normal autonomic function. However, in 17 of the 20 patients (85%) all three frequency domain parameters were abnormal. The time domain parameters PNN50 and rMSSD were abnormal in the same group of patients. When the group of patients was subdivided into intermediate dose anthracycline containing chemotherapy (regimen A) versus high dose chemotherapy (regimens B or C), no differences were observed for all (time and frequency domain) heart rate variability parameters. Comparison of irradiation of the right versus the left side of the chest wall showed no differences for any of the heart rate variability time and frequency domain parameters.

Figure 1. Impact on autonomic balance for patients (solid) versus age matched healthy controls (open) (time domain HRV parameters). SDANN, standard deviation of the averages of normal RR intervals in all 5 minute segments of a complete 24 h recording; SDNN, standard deviation of all normal RR intervals. Error bars= SD

Figure 2. Autonomic impairment after anthracycline treatment for patients with breast cancer (solid) v age matched controls (open) (time domain HRV parameters). All parameters were significantly lower for patients. PNN50, percentage of differences between successive normal RR intervals greater than 50 ms; rMSSD: the square root of the mean of the squared differences between successive normal RR intervals. Error bars = SD. * $p < 0.05$

Figure 3. Impact on autonomic balance for breast cancer patients treated with anthracyclines (solid) v age matched controls (open). (frequency domain HRV parameters). HF, high frequency; LF, low frequency; TP, total power. Error bars=SD. * $p < 0.05$

frequency domain parameters.

DISCUSSION

The results of this study show that asymptomatic breast cancer patients with normal systolic left ventricular function, treated with high dose anthracycline based chemotherapy and radiotherapy, have a high prevalence of autonomic impairment. While diastolic dysfunction was present in a large number of patients (50%), an even larger proportion (85%) of patients had autonomic abnormalities, as demonstrated by heart rate variability analysis. These findings suggest that heart rate variability analysis may be a more sensitive tool than assessment of systolic function for the early detection of anthracycline induced cardiac damage, although the specificity of abnormal heart rate variability is not known.

As anthracyclines are increasingly used in adjuvant chemotherapy regimens for breast cancer, and the indications for adjuvant chemotherapy have increased,^{340,341} the development of cardiac dysfunction is likely to be an increasingly important clinical problem in these patients.

The risk of inducing cardiac abnormalities - including the development of heart failure- even years after treatment as a result of anthracycline therapy has been recognized for a long time.^{2,3,208,351} Suitable tests are needed to adapt chemotherapy schedules and monitor supportive cardiac treatment. However, a highly sensitive screening method that is capable of predicting cardiac dysfunction is still to be found.

Currently, LVEF measured by radionuclide ventriculography or echocardiography is used to detect or exclude cardiac damage in cancer patients treated with anthracyclines. However, LVEF measurement is a relatively insensitive tool for detecting anthracycline induced cardiotoxicity at an early stage.²¹ This is largely because no considerable change in systolic function occurs until a critical amount of morphological damage has been taken place. After this point deterioration proceeds rapidly and the prognosis is poor.

In our patient group we observed a high prevalence of diastolic abnormalities, although LVEF was normal. This suggests that diastolic impairment precedes systolic dysfunction, in agreement with previous reports.^{22,255} Thus, in comparison with LVEF, the assessment of diastolic function by echocardiography may show cardiotoxicity in an earlier stage.

A striking finding in our study was the high prevalence of autonomic dysfunction, as measured by heart rate variability analysis. As in other forms of cardiac pathology, the role of autonomic changes in the pathogenesis of heart failure is increasingly recognized. Baroreceptor control of heart rate, sympathetic traffic, and heart rate variability have shown to be impaired in patients with mild or moderate heart failure.^{274,354} Heart rate variability analysis has been shown to detect left ventricular dysfunction in an early stage,^{343,344} and may also be of prognostic value in heart failure.²⁷⁴ An important marker in this respect is a reduction in parasympathetically mediated heart rate variability.³⁴²

Heart rate variability analysis in our patient group showed that the time domain parameters related to parasympathetic activity (rMSSD, PNN50) were particularly

likely to be abnormal; in addition, the frequency domain parameters showed a similar pattern of autonomic change.

These results are in agreement with the study of Postma et al,²⁴ who examined autonomic balance by heart rate variability analysis in long term survivors of malignant bone tumors treated with anthracyclines. Those patients also had particularly reduced parasympathetic activity, which correlated with the cumulative anthracycline dose.

It is generally believed that anthracyclines do not have direct toxic effects on the autonomic nervous system and thus the observed abnormalities in our study would be considered secondary to left ventricular dysfunction. It is thought that direct injury to the myocytes plays a major role in anthracycline induced cardiotoxicity. Thus the observed abnormalities in heart rate variability in our study may be secondary to myocardial damage. However, if chemotherapeutic agents have a direct effect on neurones, the changes in heart rate variability may not reflect cardiac changes alone.

Study limitations

The number of patients in this exploratory study was rather small and the group of patients was rather heterogeneous with respect to chemotherapy regimens, irradiation, and time after discontinuation of chemotherapy. Given the observational design of the study, while the data can be used to generate new hypotheses, obviously they need to be confirmed in a larger, prospective study with a homogeneous population. Such a study is needed to clarify the prognostic role of the autonomic changes and to determine whether these changes may precede the diastolic changes, as suggested by the higher prevalence of autonomic changes than diastolic abnormalities in our study. Further, since our patients were all fit and normally active, the observed abnormalities appear to be related to previous anthracycline based chemotherapy although it can not be excluded that a more sedentary lifestyle resulting from the disease may lead to some deconditioning.

Clinical implications

We studied asymptomatic patients with preserved systolic left ventricular function who had undergone anthracycline drug treatment for breast cancer. A large proportion had indications of cardiac toxicity. Careful monitoring is warranted, since symptoms might occur over time in many of these patients. Whether early pharmacological intervention might be beneficial is unknown and needs further study.

Acknowledgement

This research was supported by a grant from the University Hospital Groningen

CHAPTER 8

Evaluation of long-term cardiotoxicity after epirubicin-containing adjuvant chemotherapy and locoregional radiotherapy for breast cancer, with various detection techniques

*M.T. Meinardi¹, W.T.A. van der Graaf¹, J.A. Gietema¹, M.P. van den Berg²,
D.Th. Sleijfer¹, E.G.E. de Vries¹, J. Haaksma², F. Boomsma³, D.J. van Veldhuisen².*

Departments of Medical Oncology¹ and Cardiology²,
University Hospital Groningen; Department of Internal Medicine³,
Erasmus University Medical Center Rotterdam, The Netherlands

Submitted for publication

SUMMARY

Background. We evaluated in a cross-sectional design the cardiac function of 56 breast cancer patients 2 or more years after epirubicin-containing chemotherapy.

Methods. Patients had been treated with either 5 cycles of 5-fluorouracil, epirubicin (90 mg/m^2), and cyclophosphamide (FEC) (group I) ($n=30$) or with 4 cycles of FEC followed by high-dose cyclophosphamide, thiotepa and carboplatin (CTC) (group II) ($n=26$). Median age was 49 (range 27-58) years, median time after start of chemotherapy was 37 (range 24-79) months. The left ventricular ejection fraction (LVEF) was tested by radionuclide ventriculography. Diastolic function was echocardiographically assessed. Holter (24-h) analysis was performed to assess the heart rate variability (HRV) which reflects the autonomic nervous system activity. N-terminal Atrial Natriuretic Peptide (NT-ANP) and Brain Natriuretic Peptide (BNP) plasma levels were determined as biochemical markers of left ventricular dysfunction.

Results. Of the patients 11% had a decreased systolic function ($\text{LVEF} < 0.50$) and 38% an impaired diastolic function. HRV of the whole patient group was impaired, in particular the vagal activity, compared to healthy age-matched controls. ($P \leq 0.001$). NT-ANP was elevated in 9% of the patients, BNP in 4%. In 30% of the patients symptoms of mild CHF were present. These patients had a disturbed diastolic function and a reduced HRV compared to patients without these symptoms. No difference in cardiotoxicity was found between the treatment regimens (group I and II).

Conclusion. In a substantial part of breast cancer patients treated with a relatively low cumulative dose of epirubicin mild chronic cardiotoxicity is present. Whether this will culminate in overt CHF needs further investigation.

INTRODUCTION

Over the last decades anthracycline-containing adjuvant chemotherapy in women with high-risk breast cancer has been shown to result in a reduction of tumor recurrence and an improvement of survival rates, compared to regimens that do not contain anthracyclines.^{17,18} However, anthracycline-containing regimens can induce chronic cardiotoxicity. This feared side effect is probably related to the detrimental production of free radicals in the myocytes, resulting in cardiomyopathy, which can lead to left ventricular dysfunction and eventually to chronic heart failure (CHF).¹ Earlier studies have reported that most cases of anthracycline-related CHF in adults usually occur within one year from the last chemotherapy dose.² However, more recent studies in survivors of pediatric tumors cured with anthracycline-containing chemotherapy, have shown that CHF may not become clinical manifest until decades after chemotherapy.^{3,4,33} As a consequence of the considerable life expectancy that breast cancer patients nowadays have after anthracycline-containing adjuvant chemotherapy, attention for this late-onset CHF is becoming increasingly relevant in this patient group. In our opinion cardiotoxicity should not only be evaluated by the clinical endpoint CHF, but also by evaluation of the cardiac function using detection techniques which may allow identification of patients at risk in an early stage. Although determi-

nation of the left ventricular ejection fraction is most commonly used as a measure of anthracycline-induced cardiotoxicity in adult oncology, its sensitivity is limited probably because the systolic function is affected in a rather late stage of the disease.²¹ Diastolic dysfunction, impairment of the heart rate variability, and elevation of natriuretic peptides are other manifestations of cardiac dysfunction that may be earlier, more sensitive parameters of cardiotoxicity since they are thought to occur in an early stage of the disease.²⁰¹

In the current study we investigated in a cross-sectional design the presence of cardiac damage, by means of various detection techniques, in high-risk node-positive breast cancer patients two or more years after treatment with adjuvant chemotherapy consisting of 5-fluorouracil, epirubicin, and cyclophosphamide (FEC). Since the studied patients participated in a randomized trial comparing 5 courses of FEC with 4 courses of FEC followed by high-dose combination chemotherapy consisting of cyclophosphamide, thiotepa and carboplatin (CTC), with hematopoietic stem cell rescue, the second aim of the study was to evaluate whether high-dose chemotherapy gives rise to an increased probability of the occurrence of late cardiotoxicity compared to the standard regimen.

METHODS

Patients and treatment

Patients included in our study participated in a nation wide randomized trial evaluating two adjuvant chemotherapy regimens.³⁴⁶ Eligibility criteria for this trial were: breast cancer patients with four or more tumor-involved axillary lymph nodes (stage II and III), age ≤ 55 years and no metastases based on chest X-ray, liver ultrasound and bone scan. Patients were eligible for the trial after modified radical mastectomy or after breast conserving treatment. Patients were randomized between a standard treatment consisting of 5 cycles of FEC (group I) and a treatment consisting of 4 cycles of FEC, followed by high-dose combination chemotherapy with hematopoietic stem cell rescue (group II).

Chemotherapy

FEC chemotherapy cycles consisted of 5-fluorouracil (500 mg/m^2), epirubicin (90 mg/m^2) and cyclophosphamide (500 mg/m^2), administered three-weekly. Patients randomized for the high-dose regimen were treated after 4 courses of FEC with high-dose chemotherapy which consisted of cyclophosphamide (1500 mg/m^2), thiotepa (120 mg/m^2) and carboplatin (400 mg/m^2) (CTC) daily for four days. Reinfusion of peripheral blood stem cells was performed on day 7 after start of CTC. After bone marrow recovery patients in both groups were given tamoxifen, 40 mg/day orally for two years. In both treatment arms chemotherapy was followed by locoregional radiotherapy.

Radiotherapy

Radiation therapy was started after hematological recovery from the last chemother-

apy cycle, and was given with photons energy 6 MV and 8-12 MeV electrons. After mastectomy the target volume included the regional lymph node areas and the chest wall. The dose applied to the supraclavicular and axillary lymph node area was between the 46 and 50 Gy in 23 to 25 fractions using a linear accelerator. If the internal mammary lymph node area was also irradiated, this was performed with a combination of photons and electrons to decrease the dose to the heart. The chest wall was irradiated with electrons, 6 to 10 MeV, depending on the thickness of the chest wall, or with two tangential fields. The doses were between 40 and 50 Gy in 20 to 25 fractions over 5 weeks, 5 fractions a week. If breast-conserving therapy had been performed, the breast was treated with two tangential wedged fields in which the ipsilateral mammary lymph nodes were included. The dose applied to the whole field was 50 Gy in 25 fractions. To the area of the primary tumor an extra dose of 16 to 20 Gy in eight to ten fractions was administered with 6 MV photons. The cardiac function in these patients was evaluated in patients who were at least 2 years after the start of the chemotherapy.

Cardiac evaluation

The cardiac evaluation included a history and physical examination with special attention to signs and symptoms related to cardiac failure, which were classified according to the New York Heart Association (NYHA)-classification.³⁵⁵ Furthermore, patients underwent an electrocardiogram, radionuclide ventriculography, echocardiography, 24-h Holter monitoring for Heart Rate Variability (HRV) analysis and analysis of plasma natriuretic peptides. This study was approved by the Medical Ethical Committee of the University Hospital Groningen. All patients gave written informed consent.

Electrocardiogram

A standard 12-lead electrocardiogram was performed. The QT time was corrected for heart rate (QTc) with Bazett's formula. A QTc interval > 440 ms was considered prolonged.

Radionuclide ventriculography

Radionuclide ventriculography was performed for the determination of the left ventricular ejection fraction (LVEF). Therefore, 400 MBq Tc-99m labeled autologous red blood cells were injected and acquisition was done in 6 min with a large-field-of-view gamma camera with a low energy all-purpose parallel-hole collimator. A LVEF value between 0.50-0.55 was classified as borderline, values below 0.50 as abnormal.

Echocardiography

Echocardiograms were obtained using a Vingmed CFM 800 (Sonotron, Horten, Norway), equipped with a 3.25 MHz transducer. Gain setting was optimized to a level just below background noise. Two-dimensional images were obtained in the left ventricular parasternal long-axis and short-axis views and the apical four-chamber and two chamber long axis views.

Left ventricular end diastolic diameter (normal value 47 ± 4 mm) was measured to

test for ventricular dilatation. For the analysis of the diastolic function the early peak flow velocity / atrial peak flow velocity (E/A)-ratio (normal value >1), and the early peak flow deceleration time (DT) (normal < 220 ms) were measured.²⁶³

Heart Rate Variability analysis

HRV analysis is a well-accepted non-invasive tool to study the activity of the cardiac autonomic nervous system. HRV describes variations in the interval between consecutive heartbeats as well as the oscillations between consecutive instantaneous heart rates.³⁵⁶ For this analysis all patients underwent 24-h Holter registration, using a Marquette 8000 Holter system (Marquette Electronics, Inc., Milwaukee, WI, USA). From the 24-h Holter registration, the following time domain parameters were calculated as previously described³⁵⁶: the mean of all normal-to-normal RR (=NN) intervals, (Mean NN, ms), the standard deviation of all NN intervals in 24 hours (SDNN, ms), as measure of overall HRV; the standard deviation of the average NN intervals calculated over 5-minute segments (SDANN, ms), as measure of long-term fluctuations; the average of the 5-minute standard deviation of the NN interval calculated over 24 hours (SDNN index, ms), which measures the variability due to cycles shorter than 5 minutes; the root-mean-square successive difference of RR intervals (rMSSD, ms), a measure of vagal mediated short-term beat-to-beat variation. Tapes with in total more than 15% noise were excluded and HRV was analyzed only in those patients having a normal sinus rhythm. In our institution we have a database of normal HRV values of healthy individuals which allowed us to compare the HRV values of the patients with the HRV values of a control group consisting of healthy age-matched females.

Natriuretic peptides measurement

For the determination of N-terminal atrial natriuretic peptide (NT-ANP) and brain natriuretic peptide (BNP) blood was collected at rest into 10 ml tubes containing EDTA (19 mg). After sampling tubes were placed immediately in ice, and plasma was separated within 30 minutes, and subsequently stored at -80°C till the day of analysis. NT-ANP levels were measured using a radioimmunoassay (Biotop, Oulu, Finland) and BNP was assessed with an immunoradiometric assay (Shionoria, Osaka, Japan). The normal plasma values for NT-ANP are 150-500 pmol/l and 1-10 pmol/l for BNP.

Statistics

Mean values of 2 groups were compared with the two-tailed Student's t-test for normal distributed variables or the Mann-Whitney-U-test for variables without normal distribution. Chi-square test was used to compare prevalences between groups. Pearson's correlation coefficient or the Spearman's rank-sum test was used to test correlations between variables. A P -value < 0.05 was considered statistically significant.

RESULTS

A total of 56 patients participated in this study, 30 were treated with 5x FEC (group I) and 26 with 4 x FEC, followed by high-dose chemotherapy (group II). The median age

of all patients at the time of treatment was 46 (range 24-55) years, in group I 47 (31-55) and group II 46 (24-53) years. The median age of all patients at the time of follow-up investigation was 49 (range 27-58) years, in group I 51 (34-58) and group II 49 (27-57 years). The median time between the start of chemotherapy and the cardiac evaluation was 37 (range 24-79) months for the total group, and 38 (24-79) respectively 36 (24-57) months for group I and II. The cumulative dose epirubicin was 450 mg/m² for patients in group I and 360 mg/m² in group II. The surgical treatment consisted in 43 patients of mastectomy, and in 13 patients of breast conserving surgery. Twenty-six patients were irradiated to the left side of the chest, and 30 patients to the right side.

History and physical examination

Before the start of chemotherapy none of the patients had cardiac disease or cardiac complaints. At the time of cardiac evaluation, 17 (30%) had exertional dyspnea (NYHA class II), 7 in group I and 10 patients in group II. None of the evaluated patients had NYHA class III or IV symptoms. For the prevalence of exertional dyspnea no difference was found between group I and group II. None of the patients was anemic (mean hemoglobin level: 8.2 ± 0.6 mmol/l). None of the patients in this study was using medication for CHF. Because of hypertension three patients were using a beta-adrenergic blocker, and two patients an angiotensin-converting enzyme inhibitor. Eleven patients were still on tamoxifen at the time of cardiac evaluation. None of the evaluated patients had apparent clinical signs of CHF on physical examination.

Electrocardiography (ECG)

In 51 patients an ECG was recorded. In three cases non-specific T-wave abnormalities were found, and one patient had an incomplete left bundle branch block. A prolonged QTc interval (>440 ms) was found in 11 (20%) patients. This involved 6 of the 27 patients in group I and 5 of the 24 patients in group II.

Radionuclide ventriculography

Fifty-four of the 56 patients underwent radionuclide ventriculography for LVEF determination. Two patients refused this investigation but they did participate in the other cardiac evaluations. The results are summarized in table 1. The mean LVEF in these 54 patients was 0.57 (range 0.39–0.73). The mean LVEF was not statistically different between group I and II. Thirty-two patients (59%) had a normal LVEF (>0.55), 18 (62%) in group I and 14 (56%) in group II. Sixteen (30%) had a borderline LVEF (0.50–0.55), 9 (31%) in group I and 7 (28%) in group II. A decreased LVEF value (<0.50) was observed in 6 (11%) of the patients, 2 (7%) in group I and 4 (16%) in group II (n.s.).

Echocardiography

Good quality echocardiograms could be obtained in 53 of the 56 patients. In three patients this determination was hampered by obesity. Assessment of the diastolic function in these 53 patients revealed a mean E/A-ratio of 1.1 (range 0.7–2.5), and a mean DT of 170 (range 90–352) ms. For none of these parameters a difference was found between group I and II. The E/A-ratio was disturbed in 20 (38%) of the patients,

Table 1. Results of the cardiac evaluation using different detection techniques

Detection technique	Total ¹	Group I ²	Group II ²	P-value ³
Radionuclide ventriculography	<i>n</i> =54	<i>n</i> =29	<i>n</i> =25	
LVEF	0.57 (0.39-0.73)	0.58 ± 0.6	0.57 ± 0.9	ns
Nr. of pts. with LVEF 0.50-0.55(%)	16 (30%)	9 (31%)	7 (28%)	ns
Nr. of pts. with LVEF < 0.50 (%)	6 (11%)	2 (7%)	4 (16%)	ns
Echocardiography	<i>n</i> =53	<i>n</i> =30	<i>n</i> =23	
LVEDD (mm)	45 (32-59)	45 ± 6	45 ± 5	ns
E/A-ratio	1.1 (0.7-2.5)	1.1 ± 0.4	1.1 ± 0.3	ns
Nr. of pts. with E/A ratio < 1 (%)	20 (38%)	13 (43%)	7 (30%)	ns
DT (ms)	170 (90-352)	180 ± 49	170 ± 37	ns
Natriuretic peptides	<i>n</i> =54	<i>n</i> =30	<i>n</i> =24	
N-terminal ANP pmol/l	353 (160-910)	330 ± 120	380 ± 170	ns
N-terminal ANP > 500 pmol/l	5 (9%)	2 (7%)	3 (13%)	ns
BNP pmol/l	3.8 (0.6-51.1)	2.9 ± 2.5	5.0 ± 10.6	ns
BNP > 10 pmol/l	2 (4%)	0 (0%)	2 (8%)	ns

¹Data expressed as: mean (range); ²Data expressed as: mean ± SD; ³comparison of group I and II; LVEF = left ventricular ejection fraction; LVEDD= Left ventricular end diastolic diameter; E/A-ratio = early peak flow velocity / atrial peak flow velocity - ratio; DT = deceleration time; ANP = atrial natriuretic peptide; BNP = Brain natriuretic peptide.

13 (43%) patients in group I and 7 (30%) in group II (n.s.).

Natriuretic peptides

Natriuretic peptides could be determined in 54 patients. A mean NT-ANP of 350 (range 160-910) pmol/l and a mean BNP plasma level of 3.8 (range 0.6 – 51.1) pmol/l were found. The NT-ANP plasma level was elevated in 5 (9 %) patients, whereas BNP was elevated in 2 (4%) patients. None of the patients with elevated N-ANP, or BNP levels had an abnormal LVEF.

Heart rate variability analysis

All 56 patients underwent 24-h Holter-monitoring. For HRV analysis 6 registrations were excluded because of β -adrenergic blocker use (three patients) and because of the presence of supraventricular ectopy (three patients). The results of the HRV analysis are summarized in table 2. No difference in any of the HRV parameters could be

detected between group I and group II (results not shown). Compared to the age-matched healthy females patients had a higher mean heart rate and all HRV parameters reflecting short-term vagal mediated fluctuations, including the SDNN-index and the rMSSD, were reduced. The SDNN and SDANN, that are more related to the sympathetic activity, did not differ from the control group.

Table 2. Heart rate variability in breast cancer patients versus healthy age-matched controls

	Patients	Controls	P-value
Number	50	50	
Age (yrs)	48 ± 7	48 ± 7	ns
Heart rate (bpm)	83 ± 7	76 ± 8	< 0.0001
Mean NN (ms)	722 ± 63	799 ± 82	< 0.0001
SDNN (ms)	136 ± 32	145 ± 39	ns
SDANN (ms)	127 ± 31	127 ± 35	ns
SDNN-index (ms)	48 ± 14	59 ± 16	0.001
rMSSD (ms)	25 ± 11	36 ± 16	< 0.0001

Bpm: beats per minute; Mean NN: Mean of all NN-intervals; SDNN: standard deviation of all NN-intervals; SDANN: standard deviation of the average NN-intervals computed over 5-minute segments; SDNN-index: average of all standard deviations of NN-intervals computed over 5-minute segments; rMSSD: root mean square of successive differences of NN intervals

Correlations between the different detection techniques

No correlation was found between the LVEF value determined by radionuclide ventriculography and any of the echocardiographically assessed parameters of diastolic function, nor with the natriuretic peptides, nor with any of the HRV parameters. After adjusting for age, significant correlations were found between the E/A-ratio and HRV parameters including SDNN ($r=0.42$; $P=0.003$), SDANN ($r=0.44$; $P=0.002$) and the SDNN-index ($r=0.33$; $P=0.026$).

Left- versus right-sided radiotherapy

No difference was found in the mean LVEF between patients irradiated to the left side of the chest compared to those irradiated to the right side of the chest. The mean E/A-ratio did not differ, but patients treated with left-sided radiotherapy had a longer DT (194 ± 54 ms v. 162 ± 35 ms; $P=0.02$) compared to patients treated with right-sided radiotherapy. Between these groups no difference was found in HRV parameters, nor in the plasma levels of natriuretic peptides.

Patients with complaints of exertional dyspnea

The results of the cardiac evaluation of patients with exertional dyspnea ($n=17$ of 56; 30%) compared with the results of the patients without these complaints are summa-

rized in table 3. None of these patients received medication for CHF. Patients with complaints of exertional dyspnea had a higher body mass index than patients without these complaints (27.6 ± 4.1 v. 25.2 ± 3.1 kg/m²). There was no difference in age, or time after chemotherapy between patients with and patients without complaints of exertional dyspnea. The LVEF was not lower in patients with complaints of exertional dyspnea. However, patients with exertional dyspnea had a lower E/A-ratio and a longer DT, indicating an impaired diastolic left ventricular function. Furthermore, all HRV parameters in these patients were reduced compared to patients without these complaints.

DISCUSSION

The implication of anthracyclines in adjuvant chemotherapy regimens for breast cancer has been shown to be effective, however, concern exists about the risk of late cardiotoxicity. In this cross-sectional study we have evaluated the cardiac function of 56 high-risk breast cancer patients 24 to 79 months after start of chemotherapy that consisted of 5 cycles of FEC or 4 cycles of FEC followed by high-dose chemotherapy. In spite of the relative low cumulative dose epirubicin used in this study, namely 360-450 mg/m², we found signs of cardiotoxicity in a substantial part of the patients including a decreased systolic left ventricular function in 11% of the patients and an impaired diastolic left ventricular function in 38%. In addition, the HRV of the patients was reduced compared to healthy age-matched females. Moreover, 30% of the patients had complaints of exertional dyspnea. These patients were characterized by a worse diastolic function and an impaired HRV compared to patients without these complaints. In this study we did not find a difference in any of the cardiac parameters between the high-dose regimen and the standard regimen, despite the use of high-dose cyclophosphamide which may also have cardiotoxic side-effects and has been suggested as a risk-factor for anthracycline-cardiotoxicity.^{113,114} Our findings suggest that the high-dose regimen as used in this study did not contribute to an increase in late cardiotoxicity. This is in agreement with the data of Erselcan et al in which cardiotoxicity was evaluated with antimyosin scintigraphy in patients treated with 3 courses of FEC followed by CTC.³⁵⁷

No apparent effect could be detected of left- and right-sided irradiation of the chest, although a negative effect of irradiation on the diastolic function was suggested by the longer deceleration time found in the patients who were treated with left-sided radiotherapy. Irradiation by itself can induce various cardiac abnormalities, including pericarditis, valvular abnormalities and coronary artery disease.¹³³ Although cardiac irradiation has been suggested as an additional risk factor for the development of anthracycline-induced cardiotoxicity,^{167,175} it is questionable whether this applies also to modern irradiation techniques that makes use of smaller daily fractions and expose usually only a small volume of the heart to irradiation.³⁵⁸

The risk of late cardiotoxicity after anthracycline-containing adjuvant chemotherapy for breast cancer is still uncertain, due to a still limited follow-up and a restricted number of studies that have focussed on this issue. A recent study by Shapiro

Table 3. Patients with, versus patients without complaints of exertional dyspnea

Characteristic	Pts. with exertional dyspnea	Pts. without exertional dyspnea	P-value
Number	17	39	ns
Age (yrs)	49 ± 8	47 ± 7	ns
Time after chemotherapy (months)	35 ± 10	39 ± 10	
<i>Radionuclide ventriculography</i>			
LVEF	0.57 ± 0.8	0.58 ± 0.7	ns
<i>Echocardiography</i>			
LVEDD (mm)	45 ± 6	45 ± 5	ns
E/A-ratio	1.0 ± 0.3	1.2 ± 0.4	0.005
DT (ms)	196 ± 52	164 ± 38	0.003
<i>Heart rate variability</i>			
Heart Rate (beats/min)	85 ± 7	82 ± 8	ns
Mean NN (ms)	707 ± 65	733 ± 65	0.01
SDNN (ms)	117 ± 23	144 ± 38	0.01
SDANN (ms)	108 ± 23	134 ± 37	0.01
SDNN-index (ms)	42 ± 10	52 ± 17	0.02
rMSSD (ms)	20 ± 8	28 ± 12	0.01
<i>Natriuretic peptides</i>			
N-terminal ANP (pmol/l)	409 ± 165	338 ± 132	ns
BNP (pmol/l)	5.7 ± 12.7	3.2 ± 2.9	ns

LVEF = left ventricular ejection fraction; E/A-ratio = early peak flow velocity / atrial peak flow velocity - ratio; DT = deceleration time; Mean NN: Mean of all NN-intervals; SDNN: standard deviation of all NN-intervals; SDANN: standard deviation of the average NN-intervals computed over 5-minute segments; SDNN-index: average of all standard deviations of NN-intervals computed over 5-minute segments; rMSSD: root mean square of successive differences of NN intervals; ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide.

et al. showed that the risk of anthracycline-related cardiotoxicity was elevated in patients treated with a high-dose cyclophosphamide-doxorubicin (CA) regimen (cumulative dose doxorubicin 450 mg/m²) compared to patients treated with a low dose CA regimen (cumulative dose doxorubicin 225 mg/m²). This elevated risk was not only found within the first year after treatment, but also 1 till 10 years after treatment.¹⁶⁷ The cumulative dose epirubicin (360-450 mg/m²) that our study patients received is considered safe with respect to cardiotoxicity.^{150,213} However, also in patients treated with relatively low cumulative epirubicin doses, structural abnormalities in endomyocardial biopsies characteristic for anthracycline-cardiotoxicity, including myofibril lysis, dilatation of the sarcoplasmic reticulum and mitochondrial changes have been observed.³⁵⁹ Despite the relatively low cumulative dose epirubicin administered in the current study, we observed signs of cardiac damage. Obviously, from a study with a cross-sectional design without endomyocardial biopsies it can not be stated with certainty that the cardiac abnormalities in all patients have been caused by the chemotherapy. However, 30% of the patients experienced exertional dyspnea that was not present before chemotherapy and these patients had an impaired diastolic left ventricular function, and an impaired HRV compared to patients without these complaints. It seems therefore plausible that at least a part of the cardiac abnormalities observed has been induced by chemotherapy.

Concerning the patients with complaints of exertional dyspnea, our findings illustrate that these complaints may not always be caused by systolic dysfunction. The severity of systolic dysfunction, i.e. LVEF, has been reported to correlate only weakly with exercise capacity or symptom status.^{360,361} In the current study only one of the patients with an abnormal LVEF complained of exertional dyspnea. In addition, the average LVEF of the patients with complaints of exertional dyspnea did not differ compared to the patients without these complaints. However, in patients with complaints of exertional dyspnea we found an impaired diastolic function. It is increasingly recognized that diastolic dysfunction itself can cause symptomatic CHF in patients with preserved systolic function.^{362,363} Diastolic dysfunction can be considered as an early manifestation of anthracycline-induced cardiac damage that may precede systolic dysfunction.^{22,364} How anthracyclines affect the diastolic function of the heart is speculative. Since the myocardial relaxation during the diastole is partly an active energy consuming process,³⁶⁵ anthracycline-induced damage at the mitochondrial level of the myocytes may give rise to diastolic dysfunction. Furthermore, replacement of damaged myocytes by fibrous tissue may also affect the relaxing properties of the myocardium. Whether the patients with diastolic dysfunction in the present study will also develop systolic dysfunction in the future needs further follow-up.

HRV analysis in this study was performed to study the cardiac autonomic nervous system activity. HRV is reduced in patients with CHF from any cause and a reduced HRV is a negative predictor of impaired survival.²⁷⁴ Previously we and others observed an impairment of the autonomic function as measured by HRV after anthracycline chemotherapy,^{24,264} and recently also after paclitaxel containing chemotherapy.³⁶⁶ Comparison of the HRV of the patient group with an age-matched control-group showed an impaired HRV in the patient group, particularly in the parameters that reflect the vagal activity. Within the patient group patients with complaints of exertional

dyspnea showed the worst HRV. We found no correlation between the LVEF and HRV, but an age-independent positive correlation was found between the HRV and the diastolic function, suggesting that the reduction of HRV in these patients may be related to an impaired diastolic function.

Analysis of the natriuretic peptides showed an elevated NT-ANP in 9% of the patients and an elevated BNP level in 4%. NT-ANP is mainly secreted by the atria in response to an increased stretch as a result of an increased left-atrial pressure, whereas BNP is mainly produced by the ventricles in response to ventricular dilatation and increased wall stress. Plasma levels of both peptides are elevated in both symptomatic and asymptomatic patients with left ventricular systolic dysfunction from other causes.^{367,368} It has been suggested by some studies that natriuretic peptides may be useful early markers of anthracycline-induced cardiotoxicity.^{276,278,279} In the present study patients elevations of NT-ANP and BNP levels were not associated with systolic dysfunction. Whether these patients are at risk of developing CHF in the long-term is uncertain and needs further follow-up.

In summary, although in this study breast cancer patients were treated with relatively low cumulative doses of epirubicin, we found indications for mild chronic cardiotoxicity in a substantial part of the patients, particularly in patients with complaints of exertional dyspnea. Patients with exertional dyspnea were characterized by an impaired diastolic function and a reduced HRV. Patients with these complaints might benefit from medication such as angiotensin-converting enzyme inhibitors, although this remains to be proven. Whether the affected patients do have an increased risk for severe CHF in the long-term needs further follow-up.

Acknowledgements

This study was supported by a grant from the University Hospital Groningen.

CHAPTER 9

Prospective evaluation of early cardiac damage induced by epirubicin-containing adjuvant chemotherapy and locoregional radiotherapy in breast cancer patients

*M.T. Meinardi¹, D.J. van Veldhuisen², J.A. Gietema¹, W.V. Dolsma³, F. Boomsma⁴,
M.P. van den Berg², C. Volkers², J. Haaksma², E.G.E. de Vries¹, D.Th. Sleijfer¹,
W.T.A. van der Graaf¹*

Departments of Medical Oncology ¹, Cardiology ², Radiation Oncology ³,
University Hospital Groningen; Department of Internal Medicine ⁴,
Erasmus University Medical Center Rotterdam, The Netherlands

Journal of Clinical Oncology, in press

SUMMARY

Purpose. The aim of this study was to evaluate prospectively the cardiotoxic effects of epirubicin containing adjuvant chemotherapy in breast cancer patients.

Patients and methods. Patients (median age 46 years, range 28-55 years) were treated with 5 cycles of 5-fluorouracil, epirubicin (90 mg/m²), and cyclophosphamide (FEC) (group I) (n=21) or with 4 cycles of FEC followed by high-dose chemotherapy consisting of cyclophosphamide, thiotepa and carboplatin (CTC) (group II) (n=19), in both groups followed by locoregional radiotherapy. Cardiac evaluation was performed before the start of chemotherapy (T0), 1 month after chemotherapy (T1), 1 month after radiotherapy (T2) and 1 year after start of chemotherapy (T3). Cardiac evaluation consisted of determination of: the left ventricular ejection fraction (LVEF) by radionuclide ventriculography; echocardiographic determination of the diastolic function, 24-hour ECG registration for heart rate variability (HRV) analysis to assess the autonomic function; assessment of the QTc and determination of N-terminal Atrial Natriuretic Peptide (NT-ANP) and Brain Natriuretic Peptide (BNP) as biochemical markers of cardiac dysfunction.

Results. None of the patients developed overt congestive heart failure. In the total group the mean LVEF declined from 0.61 at T0 to 0.54 at T3 ($p=0.001$), resulting in a LVEF below 0.50 (range 0.42-0.49) in 17% of the patients, whereas 28 % had a decline of more than 0.10. Plasma NT-ANP levels increased gradually from 237 pmol/l at T0 to 347 pmol/l at T3 ($p<0.01$) whereas plasma BNP levels increased from 2.9 pmol/l to 5.1 pmol/l ($p=0.04$). Mean QTc increased from 406 ms at T0 to 423 ms at T3 ($p<0.01$). No persistent alterations were found in the diastolic function and HRV.

Conclusion. Treatment with relatively low doses epirubicin in adjuvant chemotherapy for breast cancer results in mild subclinical myocardial damage, as demonstrated by a decline in LVEF, an increase in the levels of natriuretic peptides and an increase in QTc. These changes may have no serious consequences in the short-term but in the long-term some patients may be at an increased risk for development of congestive heart failure.

INTRODUCTION

Anthracyclines such as doxorubicin and epirubicin, nowadays increasingly used in the adjuvant treatment, have improved the prognosis of patients with locally advanced breast cancer.^{17,18} Attention for long-term side effects of the treatment is therefore becoming more important. The most serious side effect of anthracyclines is chronic cardiotoxicity, which can culminate in congestive heart failure (CHF).³¹ Although the majority of cases of CHF becomes clinical overt within the first year after discontinuation of chemotherapy,² in some patients this may take many years.^{3,33} The risk of the late-onset CHF in breast cancer patients treated with anthracyclines is uncertain because of the still limited follow-up of these patients. Prospective evaluation of the cardiac function may help to identify patients at risk in an early stage and provides information on the risk of CHF in the long-term. Anthracycline cardiotoxicity is most

commonly assessed by determination of the left ventricular ejection fraction (LVEF) by radionuclide ventriculography, although its sensitivity and specificity for early detection of cardiotoxicity is limited.²¹ Since in the development to CHF several other physiological changes occur that may precede or coincide systolic dysfunction, assessment of these changes may be useful to detect cardiotoxicity probably in an earlier stage.²⁰¹

The aim of the current study is to evaluate prospectively the cardiotoxic effects of epirubicin containing adjuvant chemotherapy in breast cancer patients by means of various detection techniques. In addition to the assessment of the LVEF, echocardiography was performed for the determination of various cardiac parameters including the diastolic function. This is of interest since diastolic dysfunction is thought to precede systolic dysfunction.^{22,23,255} Furthermore, heart rate variability (HRV) analysis was studied as measurement of the assessment of the autonomic activity. HRV is reduced in patients with heart failure from any cause, and this reduction of HRV also occurs in an early stage of CHF.^{274,344} Furthermore, the plasma levels of the natriuretic peptides N-Terminal Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP) were determined. These biochemical markers of cardiac dysfunction are secreted by the atria respectively the ventricles in response to an increased wall pressure.³⁶⁸ An electrocardiogram was obtained to investigate the usefulness of the QT time corrected for heart rate (QTc), since prolongation of the QTc has been suggested as a measure of anthracycline-induced cardiotoxicity.²⁴⁴

METHODS

Patients and treatment

Between september 1997 and june 1999 all consecutive patients who participated in a nation wide randomized trial evaluating two adjuvant chemotherapy regimens,³⁴⁶ were also asked for prospective evaluation of the cardiac function. Eligibility criteria for the randomized trial were: breast cancer with four or more tumor-involved axillary lymph nodes (stage II and III), age ≤ 55 years and no metastases based on chest X-ray, liver ultrasound and bone scan. Patients were eligible for the trial after modified radical mastectomy or after breast conserving treatment. Patients were randomized between a control treatment arm consisting of 5 cycles of FEC (group I) and an arm containing 4 cycles of FEC, followed by high-dose combination chemotherapy with hematopoietic stem cell rescue (group II).

Chemotherapy

FEC chemotherapy cycles consisted of 5-fluorouracil (500 mg/m²), epirubicin (90 mg/m²) and cyclophosphamide (500 mg/m²), administered three-weekly. Patients randomized for the high-dose regimen were treated after 4 courses of FEC with high-dose chemotherapy which consisted of cyclophosphamide (1500 mg/m²), thiotepa (120 mg/m²) and carboplatin (400 mg/m²) (CTC) daily for four days. Reinfusion of peripheral blood stem cells was performed on day 7 after the start of CTC. After bone marrow recovery patients in both groups were given tamoxifen, 40 mg/day orally for

two years.

Radiotherapy

In both treatment arms chemotherapy was followed by locoregional radiotherapy. Radiation therapy was started after hematological recovery from the last chemotherapy cycle, and was administered with photons energy 6 MV and 8-12 MeV electrons. After mastectomy the target volume included the regional lymph node areas and the chest wall. The dose applied to the supraclavicular and axillary lymph node area was between the 46 and 50 Gy in 23 to 25 fractions using a linear accelerator. If the internal mammary lymph node area was also irradiated, this was performed with a combination of photons and electrons to decrease the dose to the heart. The chest wall was irradiated with electrons, 6 to 10 MeV, depending on the thickness of the chest wall, or with two tangential fields. The doses were between 40 and 50 Gy in 20 to 25 fractions over 5 weeks, 5 fractions a week. If breast-conserving therapy had been performed, the breast was treated with two tangential wedged fields in which the ipsilateral mammary lymph nodes were included. The dose applied to the whole field was 50 Gy in 25 fractions. To the tumor bed a boost of 16 to 20 Gy in eight to ten fractions was administered with 6 MV photons.

Cardiac evaluation

The cardiac evaluation was performed at the following points of time: before start of chemotherapy (T0), one month after chemotherapy but before radiotherapy (T1), one month after completion of the radiotherapy (T2) and one year after the start of chemotherapy (T3). The cardiac evaluation included a history and physical examination with special attention to signs and symptoms related to CHF. At all four points of time the cardiac function was assessed by the following techniques: echocardiography, a 24-h Holter monitoring for HRV analysis, determination of plasma natriuretic peptides, and electrocardiography. At T0 and T3 patients also underwent a radionuclide ventriculogram. The study was approved by the Medical Ethical Committee of the University Hospital Groningen, the Netherlands. All patients gave written informed consent.

Radionuclide ventriculography

Radionuclide ventriculography was performed for the determination of the left ventricular ejection fraction (LVEF). Therefore 400 MBq Tc-99m labeled autologous red blood cells were injected and acquisition was performed in 6 min with a large-field-of-view gamma camera with a low energy all-purpose parallel-hole collimator. A LVEF less than 0.50 was considered abnormal. A decrease in LVEF of more than 0.10 was considered to indicate significant cardiotoxicity.

Echocardiography

Echocardiograms were performed using a Vingmed CFM 800 (Sonotron, Horten, Norway), equipped with a 3.25 MHz transducer. Gain setting was optimized to a level just below background noise. Two-dimensional images were obtained in the left ventricular parasternal long-axis and short-axis views and the apical four-chamber and two

chamber long axis views.

For the analysis of diastolic function the following parameters were measured: early peak flow velocity / atrial peak flow velocity (E/A)-ratio (normal value > 1), the deceleration time of the early peak flow (DT) (normal value < 220 ms) and the isovolumic relaxation time (IVRT) (normal value < 100 ms). Left ventricular end diastolic diameter (normal value 47 ± 4 mm) was measured to test for ventricular dilatation.²⁶³

Heart Rate Variability analysis

HRV analysis, a well-accepted non-invasive tool to study the activity of the cardiac autonomic nervous system measures the variations in the interval between consecutive heartbeats.³⁵⁶ For HRV analysis all patients underwent 24-h Holter registration, using a Marquette 8000 Holter system (Marquette Electronics, Inc., Milwaukee, WI, USA). From the 24-h Holter registration, the following time domain parameters were calculated as previously described³⁵⁶: the standard deviation of all normal intervals (NN) in 24 h (SDNN, ms); the standard deviation of the average NN intervals calculated over 5-min segments (SDANN, ms); the average of the 5-min standard deviation of the NN interval calculated over 24 h (SDNN index, ms); the root-mean-square successive difference of RR intervals (rMSSD, ms). Frequency domain analysis was performed by using discrete Fourier Transformation. Low frequency power (LF; 0.04-0.15 Hz), high frequency power (HF; 0.15- 0.4 Hz) and Total Power (TP; 0.0033-0.4 Hz) were calculated. Of LF, HF and TP the square root was taken to obtain a normal distribution, and therefore these variables are presented in ms (as opposed to ms²). Tapes with more than 15 % noise or ectopy were excluded and HRV was analyzed only in those patients having a normal sinus rhythm.

Electrocardiogram

A standard 12-lead electrocardiogram was recorded. The QT time was corrected for heart rate (QTc) with Bazett's formula ($QTc = QT/\sqrt{RR}$). A QTc time more than 440 ms was considered prolonged.

Natriuretic peptides

For the determination of N-Terminal ANP (NT-ANP) and BNP blood was collected at rest into 10 ml tubes containing EDTA (19 mg). After sampling, tubes were placed immediately in ice, and plasma was separated within 30 minutes, and subsequently stored at -80°C till the day of analysis. NT-ANP levels were measured using a radio-immunoassay (Biotop, Oulu, Finland) and BNP was assessed with an immunoradiometric assay (Shionoria, Osaka, Japan). The normal plasma values for NT-ANP are 150-500 pmol/l and for BNP 1-10 pmol/l.

Statistics

Quantitative variables were compared between two groups using an unpaired t-test for normally distributed variables or Wilcoxon two-sample test for skewedly distributed variables. For comparisons between more than two groups, an F-test or a Kruskal-Wallis test was used as appropriate. Normally distributed variables are reported as mean $\pm$ SD. Skewedly distributed variables are reported as median and

range (min-max). A 3-way ANOVA model was constructed to evaluate the effect of heart frequency on the E/A-ratio. By means of a 2-way ANOVA model the course of cardiac parameters over the points of time were compared between patients who had a LVEF decline of more than 0.10 or a LVEF of less than 0.50 at T3 versus the patients with a decline of less than 0.10 and a LVEF of more than 0.50 at T3. Correlations between variables were calculated using Pearson's or Spearman correlation coefficient test as appropriate. All p-values are two-sided and a p-value < 0.05 was considered statistically significant. To correct for multiple comparisons, a Bonferroni correction was used. SAS version 6.12 (Cary, NC) was used for all statistical evaluations.

RESULTS

In total 50 consecutive patients were asked for the cardiac evaluation of whom 40 agreed to participate. A total of 40 patients participated in this study, 21 were treated with 5x FEC (group I) and 19 with 4 x FEC, followed by high-dose chemotherapy (group II). The cumulative dose epirubicin in group I was 450 mg/m² and in group II 360 mg/m². The median age of all patients at the time of treatment was 46 (range 28-55) years. No difference was found in age between group I and II (group I: 48 (28-55) years vs. group II: 46 (35-53) years; p=NS).

In group I 15 of the 21 (71%) patients were treated with mastectomy and 6 (29%) with breast conserving surgery, whereas in group II 18 of the 19 (94%) patients were treated with mastectomy and 1 (6%) patient with breast conserving surgery. In group I 13 of the 21 (62%) patients were irradiated to the left side of the chest, and 8 (38%) to the right side. In group II 12 of the 19 (63%) patients were irradiated to the left side of the chest, and 7 (37%) to the right side. None of the patients was known to have cardiac disease at the start of chemotherapy. Two patients had long-standing hypertension for which they were treated with an angiotensin converting enzyme inhibitor and a calcium-blocker respectively. Two patients did not complete the cardiac evaluation because of tumor recurrence. Of these one patient participated only at T0 and T1, whereas the other participated at T0, T1 and T2. Furthermore, some data are missing due to logistic or technical reasons.

Cardiac evaluation

None of the patients developed clinically apparent CHF within the first year after treatment.

Systolic and diastolic blood pressures did not change from T0 to T3 and no differences were found between group I and II. The mean systolic blood pressure for the whole group at T0 was 127 ± 14 mmHg vs. 125 ± 14 mmHg at T3 (p=NS), whereas the diastolic blood pressure at T0 was 80 ± 7 mmHg vs. 78 ± 10 mmHg at T3 (p=NS). Table 1. summarizes the results of the cardiac evaluation at T0 and T3.

Radionuclide ventriculography

In 39 patients a LVEF determination was performed at T0 and in 35 patients at T3. The

Table 1. Cardiac function of breast cancer patients before and one year after chemotherapy

Detection technique	Total		group I		group II	
	T0	T3	T0	T3	T0	T3
Radionuclide ventriculography						
Left ventricular ejection fraction	0.61 ± 0.08	0.54 ± 0.06**	0.63 ± 0.07	0.55 ± 0.06**	0.59 ± 0.07	0.53 ± 0.06*
Echocardiography						
E/A-ratio	1.24 ± 0.26	1.17 ± 0.26	1.25 ± 0.31	1.23 ± 0.27	1.23 ± 0.2	1.11 ± 0.25
Deceleration time (ms)	176 ± 39	187 ± 36	180 ± 43	195 ± 40	171 ± 35	177 ± 29
Isovolumic relaxation time (ms)	82 ± 14	79 ± 13	84 ± 15	80 ± 12	78 ± 14	77 ± 14
Heart Rate Variability						
SDNN (ms)	135 ± 37	134 ± 40	136 ± 34	141 ± 33	133 ± 41	124 ± 47
SDANN (ms)	121 ± 37	129 ± 36	123 ± 35	129 ± 31	119 ± 40	127 ± 43
SDNN-index (ms)	55 ± 15	50 ± 15	56 ± 16	52 ± 15	53 ± 15	48 ± 15
RMSSD (ms)	30 ± 14	27 ± 13	30 ± 14	28 ± 12	29 ± 14	26 ± 14
SQRT Total Power (ms)	35 ± 11	32 ± 11	36 ± 12	34 ± 12	34 ± 11	30 ± 10
SQRT Low Frequency (ms)	23 ± 8	21 ± 8	24 ± 8	23 ± 9	22 ± 7	19 ± 7
SQRT High Frequency (ms)	12 ± 6	11 ± 5	13 ± 6	11 ± 5	12 ± 6	10 ± 6
Heart Rate (beats/min)	79 ± 7	79 ± 7	78 ± 7	77 ± 7	79 ± 7	82 ± 7
Electrocardiography						
QTc (ms)	406 ± 11	423 ± 16**	406 ± 10	422 ± 15**	406 ± 11	424 ± 18**
Natriuretic peptides						
NT-ANP (nmol/l)	237 ± 76	347 ± 106**	229 ± 72	334 ± 79**	245 ± 81	361 ± 131*
BNP (pmol/l)	2.9 ± 2.8	5.1 ± 4.3**	3.2 ± 3.1	5.4 ± 3.2	2.6 ± 2.3	4.8 ± 5.4

T0 = before start of chemotherapy; T3 = one year after start of chemotherapy; *significant change between T0 and T3, p-values < 0.05; ** idem, p-value < 0.01;

E/A-ratio= early peak flow velocity / atrial peak flow velocity - ratio; For abbreviations of HRV parameters, see text; SQRT= square root;

ANP = atrial natriuretic peptide; BNP= brain natriuretic peptide.

mean LVEF for the total group at T3 was lower compared to T0 (T0: 0.61 ± 0.08 vs. T3: 0.54 ± 0.06 ($p=0.001$)). Between group I and II no difference was found in the mean decline in LVEF. At T3 six (17%) patients had a LVEF less than 0.50 (range 0.42-0.49), although all had a normal LVEF at T0. Two of these six patients were in group I and 4 in group II. Ten (28%) patients had a decline of more than 0.10 (range 0.10-0.22), however 6 patients still had a LVEF at T3 above 0.50. Of these ten patients five were in group I and five in group II.

Echocardiography

Good quality echocardiograms could be obtained in 36 patients at T0, in 35 patients at T1, in 34 patients at T2, and in 36 patients at T3. At T3 no differences were observed in the mean values of the diastolic function parameters compared to the mean values at T0. This applied to both the total group and to the groups separately. In group I the mean E/A, the DT and the IVRT did not change over the different points of time. However, in group II the mean E/A-ratio at T1 was decreased compared to T0 (T0: 1.23 ± 0.20 vs. 1.00 ± 0.25 ; $p=0.014$), but gradually increased after T1. However, in a 3-way ANOVA model in which was corrected for the heart frequency, no change in E/A-ratio over time was found. The other parameters in group II, the mean DT and IVRT, did not change over time.

Heart rate variability analysis

Successful HRV registration could be performed in 37 patients at T0, 37 at T1, 35 at T2 and 35 at T3. The changes in HRV and the mean heart rate over 24 h (HR) over the 4 points of time are also illustrated in figure 1. In this figure the square root of Total Power, which is a measure of overall HRV, is shown. At T3 no difference was found in the HRV parameters and HR compared to T0. Furthermore, in group I the HRV parameters and HR did not change during time. However, in group II all HRV parameters were reduced at T1, compared to T0 (p -values < 0.01), but returned to baseline values at T2 and T3, while the mean HR in group II increased from 79 ± 7 beats/minute (bpm) at T0 to 88 ± 8 bpm at T1 ($p < 0.001$), whereafter it decreased to 84 ± 9 bpm at T2 and, to 82 ± 7 bpm at T3 which was not different from the HR at T0.

Electrocardiography

In total 37 ECGs were performed at T0, 35 at T1, 35 at T2 and 35 at T3. At T3 the mean QTc time of the total group was significantly prolonged compared to T0 (T0: 406 ± 11 ms vs. T3: 423 ± 16 ms; $p<0.001$). Between group I and II No difference was found in the mean increase in QTc time from T0 to T3. At T1 the mean QTc time of the total group (420 ± 11 ms) was already increased compared to T0 ($p=0.006$), and was 426 ± 18 ms at T2. From T1 to T3 no significant change was observed. Between group I and II no differences in the mean QTc time were found at the different points of time. The QTc time was increased (> 440 ms) in none of the patients at T0, in 4 (11%) patients at T1 in 5 (17%) patients at T2 and in 6 (17%) patients at T3.

Figure 1. Differences between group I and II in HRV reflected as $\sqrt{\text{Total Power (TP)}}$ and heart rate (HR) in the course of time. Data presented as mean $\pm$ SD, ms= milliseconds; bpm: beats/min. * Significant difference between T0 and T1 in group II; $p < 0.001$. T0= before start chemotherapy; T1= one month after chemotherapy; T2= one month after radiotherapy; T3= one year after start of chemotherapy

Natriuretic peptides

Natriuretic peptides were determined in 39 patients at T0, 39 patients at T1, 36 patients at T2 and 36 patients at T3. The changes in natriuretic peptides in the course of time are presented in figure 2. At T3 the mean NT-ANP plasma level of the total group was significantly higher compared to T0 (T0: 237 ± 76 pmol/l vs. T3: 347 ± 106 pmol/l; $p < 0.001$). Between group I and II no difference was found in the mean increase in NT-ANP from T0 to T3. The mean NT-ANP of the total group at T1 (297 ± 150 pmol/l) was already increased compared to T0 ($p = 0.048$), but the mean NT-ANP at T2 (297 ± 109 pmol/l) was not higher compared to T1, while the increase from T2 to T3 was not significant ($p = 0.054$). Between group I and II no difference was found in the mean NT-ANP level at the different points of time.

At T3 the mean BNP plasma level of the total group was significantly higher compared to T0 (T0: 2.9 ± 2.8 pmol/l vs. T3: 5.1 ± 4.3 pmol/l; $p = 0.04$). Between group I and group II no difference was found in the increase in BNP. At T1 the mean BNP plasma level of the total group was 4.2 ± 3.5 pmol/l, which was higher compared to T0 ($p = 0.048$). At T2 the mean BNP level was 3.0 ± 3.0 pmol/l, not different from T1. From T2 to T3 the BNP level increased ($p = 0.002$). Between group I and group II no difference was observed in the mean BNP level at the different points of time.

Comparisons of detection techniques

Early changes between T0 and T1 in the plasma levels of the natriuretic peptides, in the QTc time, HRV and HR and diastolic function parameters did not correlate with

Figure 2. Alterations in NT-ANP and BNP plasma levels in the first year following chemotherapy. Data presented as mean $\pm$ SD. T0= before start chemotherapy; T1= one month after chemotherapy; T2= one month after radiotherapy; T3= one year after start of chemotherapy

the decline in LVEF between T0 and T3. Except for the change in DT between T0 and T3, which correlated inversely ($r=-0.49$, $p<0.01$), none of the changes in the other parameters studied, was associated with the change in LVEF.

A subanalysis was performed between the patients who had developed a decline in LVEF of more than 0.10 or a LVEF of less than 0.50 at T3 (group A), versus the patients with a decline of less than 0.10 and a LVEF of more than 0.50 at T3 (group B). This analysis did not show differences in the diastolic function parameters E/A-ratio and IVRT, but the DT increased more in group A patients. Whereas at T0 the DT was not different between these groups (group A: 169 ± 37 ms vs. group B: 189 ± 38 ms; $p=NS$), at T3 patients in group A had a higher DT compared to patients in group B (209 ± 42 ms vs. 176 ± 30 ms; $p=0.012$).

The mean HR between group A and B was not different at T0 (group A: 77 ± 8 bpm vs. 80 ± 6), but at T1 the mean HR was higher in group A (group A: 89 ± 6 bpm vs. group B: 81 ± 10 bpm; $p=0.024$), while the Hb level was not different (group A: 6.8 ± 0.8 mmol/l vs. group B: 6.6 ± 0.7 mmol/l; $p=NS$). This difference was no longer observed at T2 (group A: 83 ± 9 bpm vs. group B: 80 ± 8 bpm; $p=NS$) nor at T3 (group A: 80 ± 5 bpm vs. group B: 79 ± 8 bpm; $p=NS$). Between group A and B no difference was found in the HRV, QTc time and natriuretic peptides at the various points of time.

Left-sided versus right-sided radiotherapy

Between patients irradiated to the left versus those irradiated to the right side of the chest, no differences were detected in any of the tested cardiac parameters.

DISCUSSION

Anthracyclines are increasingly used as part of adjuvant chemotherapy regimens for

locally advanced breast cancer.^{17,18} However, concern exists that in the long term the survival benefit is counterbalanced by chronic cardiotoxicity. The risk of anthracycline induced congestive heart failure (CHF) depends on the cumulative dose, and for epirubicin the estimated risk has been reported to be less than 1% below 550 mg/m², but it increases to 4% at 900 mg/m² and to 15% at 1000 mg/m².²¹³ However, the prevalence of subclinical cardiotoxicity is probably much higher. In the current prospective study we found during the first year after chemotherapy a mild but significant impairment of the cardiac function in breast cancer patients treated with relatively low cumulative doses of epirubicin (360–450 mg/m²). This impairment was most clearly indicated by a decline in the mean left ventricular ejection fraction (LVEF) from 0.61 to 0.54, which resulted in 17% of the patients in an ultimate LVEF below 0.50. In addition, plasma N-Terminal atrial natriuretic peptide (NT-ANP) and brain natriuretic peptide (BNP) levels increased in the course of time and a prolongation of the QTc time was observed. We found no indications for an increase in cardiotoxicity by irradiation nor by high-dose cyclophosphamide, both factors having been suggested to increase the risk of cardiotoxicity.

The observed drop in LVEF is a clear indication that even low dosages epirubicin-containing adjuvant chemotherapy cause impairment of the systolic function as a result of myocardial damage. The magnitude of the mean decline in LVEF found in our study is somewhat greater than observed by Cottin et al. who found in 61 breast cancer patients treated with a cumulative dose epirubicin of 438 ± 96 mg/m² a decrease in the mean LVEF from 0.58 to 0.55 twelve months after chemotherapy.³⁶⁹ Although the mean LVEF in the present study remained within the normal range, 17% of the patients had an ultimate LVEF below 0.50 (ranging from 0.42 to 0.49) and 28 % of the patients had a decline in the LVEF of more than 0.10. Particularly patients with a LVEF below 0.50 might be at an increased risk for the development of CHF in the long-term. On the other hand, an absolute decrease in LVEF of more than 0.10 even if LVEF remains within the limits of normal, may also be a risk factor. Whether the observed deterioration in cardiac function is a stable process or slowly progressive, remains to be determined by further follow-up.

Although LVEF determination is most commonly used by oncologists, its sensitivity and specificity for early detection of cardiotoxicity has been shown to be limited.²¹ We therefore evaluated the usefulness of several other detection techniques, which may be more indicative for subclinical cardiotoxicity. Natriuretic peptides have been shown to be useful markers of left ventricular dysfunction in both symptomatic and asymptomatic patients.³⁷⁰ Previous studies evaluating their value in the detection of anthracycline cardiotoxicity suggested that the levels of natriuretic peptides become elevated before the development of CHF and even before a decline in LVEF occurs.^{276,279} The increase in NT-ANP, which was already present one month after completion of chemotherapy, developed progressively over the study time, suggesting that the atrial pressure increases. However, the mean NT-ANP levels remained within the normal range while only 2 patients had an NT-ANP level above the normal values after one year. BNP levels also increased, reflecting an increase in ventricular pressure. In the current study the increased level of natriuretic peptides was not associated with a decrease in LVEF. Since none of our patients developed CHF the predictive value of

the elevations of NT-ANP and BNP levels remains as yet uncertain.

Several studies have shown that besides systolic dysfunction anthracyclines also induce diastolic dysfunction at an early stage.^{22,23,255} We found no evident signs of diastolic dysfunction as measured by echocardiography. However, in a subgroup of patients with a decline in LVEF of more than 0.10 and/or a LVEF of less than 0.50, we found a higher increase in the deceleration time than in the other patients, indicating an impaired relaxation of the left ventricle. Because we were not able to find changes in the other diastolic function parameters, the significance of this finding is uncertain.

Previously we and others observed a reduction of HRV after anthracycline chemotherapy.^{24,264} In the current study no persistent reduction of HRV was demonstrated. However, particularly the measurement of HRV shortly before start of chemotherapy may have resulted in a decreased HRV due to mental stress.³⁷¹ This initial decrease may have masked a reduction of HRV due to epirubicin cardiotoxicity.

The QTc time was also determined since it has been suggested to be a sign of cardiotoxicity.^{244,301} We observed a persistent increase in the mean QTc which was already present after completion of chemotherapy, and which resulted in a significantly prolonged QTc (>440 ms) in 17% of the patients after one year. Although prolongation of the QTc may be multifactorial, it could indicate a disturbance in the repolarization of the myocardium as a result of myocardial damage. The clinical significance of this finding is uncertain, but a prolonged QTc may predispose patients to the development of arrhythmias.³⁷² Ferrari et al. showed that prolongation of the QTc was more often found in patients treated with higher doses of anthracyclines, but they found no correlations between prolonged QTc and functional echocardiographic alterations.²⁴⁵ We also observed no correlation between the increase in QTc time and the decline in LVEF.

No indications were found for an enhancement of cardiotoxicity by the use of left-sided irradiation of the chest compared to right-sided irradiation, although this study was not designed to address them. Cardiac irradiation as applied in the treatment of breast cancer has been suggested to be an additional risk factor for the development of anthracycline-induced cardiotoxicity.^{27,167,373} However, with the development of the currently used modern irradiation techniques, which make use of small daily fractions and sophisticated planning techniques resulting in low cardiac dose volumes, this risk has been reduced considerably. No significant increase in cardiotoxicity was found as a consequence of the high-dose cyclophosphamide containing chemotherapy, although cyclophosphamide has been suggested as an additional risk factor of anthracycline cardiotoxicity.¹³⁰ Erselcan et al. could also not demonstrate an enhancement of subclinical cardiotoxicity as a consequence of high-dose cyclophosphamide following epirubicin-containing adjuvant chemotherapy.³⁵⁷

In summary, we observed several indications of (mild) cardiotoxicity in breast cancer patients following adjuvant chemotherapy which contained relatively low cumulative doses of epirubicin. This was shown by a decrease in LVEF, and additionally by an increase of the plasma levels of the natriuretic peptides as well as by an increase in the QTc time. Because these changes are already observed after low doses of anthracyclines and shortly after chemotherapy, they suggest that the extent of subclinical cardiotoxicity after treatment with anthracyclines is probably higher than previ-

ously described. The observed degree of subclinical cardiotoxicity may be no serious threat in the short-term. However, in the long-term a part of these patients may be at increased risk of developing late-onset cardiac dysfunction, which is especially relevant in patients with a favorable prognosis.

CHAPTER 10

FUTURE PERSPECTIVES

The follow-up study of survivors of metastatic testicular cancer presented in this thesis indicated that these patients are at an increased risk of cardiovascular disease. Just recently our observation has been confirmed by a large study in 946 long-term survivors of testicular cancer from the Royal Marsden Hospital in London. They presented their data at the American Society of Clinical Oncology meeting 2000 and found cardiovascular events in 7.8 % of the cured patients after chemotherapy. The risk for cardiac events in cured disseminated patients was increased when compared with testicular cancer patients who were in surveillance and did not receive additional treatment after orchidectomy.³⁷⁴

After establishing the definite prevalence and pathogenesis of this chronic side effect of cisplatin-based chemotherapy, several ways to prevent this side effect should be considered. Elimination of the responsible compound (e.g. cisplatin or bleomycin) or replacing it by a less toxic analogue appears not a feasible approach. Replacement of cisplatin by carboplatin, or omitting bleomycin does indeed reduce toxic side effects but this is at the cost of less anti-tumor efficacy.^{195,197} Therefore, it seems likely that in the treatment of metastatic testicular cancer four cycles of bleomycin, etoposide and cisplatin will remain the standard regimen, although in patients with a good prognosis three cycles of this combination may also be sufficient.³⁷⁵ Therefore elucidating the pathogenesis behind the increased risk of cardiovascular disease in survivors of metastatic testicular cancer will be essential and may result in other methods to prevent or ameliorate chronic cardiovascular toxicity.

Endothelial dysfunction is nowadays thought to play a key role in the development of cardiovascular disease in general.³⁷⁶ Endothelial dysfunction in survivors of metastatic testicular cancer treated with chemotherapy might be induced directly by chemotherapy or might be induced by secondary changes such as dyslipidemia. To investigate the pivotal role of endothelial dysfunction, its prevalence has to be further evaluated in testicular cancer patients after chemotherapy and should be compared with the prevalence in both testicular patients treated with orchidectomy only and in the normal healthy population. Also prospective evaluation of the endothelial function, before, during and after chemotherapy is required to test the hypothesis that endothelial dysfunction is involved. Besides various biochemical parameters (e.g. von Willebrand factor, thrombomodulin, intercellular adhesion molecules), and measurement of microalbuminuria, also other non-invasive functional tests such as echographic measurement of changes in the forearm blood flow before and after vasodilating agents might be useful for this purpose. Besides *in vivo* studies, also *in vitro* experiments using cultured endothelial cells may help to gain understanding of the effects of chemotherapy on the function of endothelium at the cellularly and molecularly level. If future studies indicate a direct induction of endothelial dysfunction by chemotherapy, prevention of endothelial dysfunction during chemotherapy should be considered. Possibly a cytoprotective agent such as amifostine may be capable of reducing the damaging effect of chemotherapy on the endothelium and therefore influence long-term side effects.

Whether gonadal toxicity caused by the same cytostatic agents plays a role in the development of cardiovascular toxicity should also be investigated. We found that subclinical compensated hypogonadism was more pronounced in survivors of tes-

ticular cancer treated with chemotherapy compared to those treated with orchiectomy only. In addition chemotherapy treated patients with lower plasma levels of testosterone were more insulin resistant. Subclinical compensated hypogonadism might have an adverse effect on the cardiovascular risk-profile and possibly correction of subclinical hypogonadism might therefore be another approach to reduce the risk of cardiovascular disease.³⁷⁷

Persistent hypomagnesemia is another mechanism that might be involved in the development of cardiovascular disease as has been described in this thesis. Therefore future studies should also explore whether strict correction of magnesium during and after chemotherapy might prevent the development of an adverse cardiovascular risk profile.

Further evaluation of chronic cardiotoxicity in breast cancer patients treated with anthracycline containing adjuvant chemotherapy remains an important issue in the forthcoming years. The prevalence of this side effect in the long-term is currently insufficiently known because of the currently limited follow-up duration.

If the prevalence of chronic anthracycline-related cardiotoxicity in the long-term will appear to be higher than expected, ways to prevent this side effect will have to be explored. It should be questioned whether the more effective cancer treatment with anthracycline is counterbalanced by its chronic cardiotoxicity. If the prevalence of chronic cardiotoxicity is considered to be not acceptable, eliminating the anthracyclines from the adjuvant regimens is a theoretical possibility. However, it is more likely that other ways of prevention will be followed. The search for a less cardiotoxic anthracycline analogue remains important. Epirubicin is such an analogue which is currently widely applied, but at higher doses it is also cardiotoxic and is therefore not yet the ideal analogue.²¹³ Also new ways of administration such as the encapsulation of anthracyclines into liposomes seem to be a promising approach to reduce the cardiotoxicity,¹⁹⁶ but this treatment has been insufficiently evaluated and is quite expensive.

Much knowledge on the pathogenesis of anthracycline cardiotoxicity has been gathered over the last decades. The formation of free oxygen radicals in the myocytes during anthracycline administration appears to play a key role. Scavenging these radicals can be an effective way to prevent cardiotoxicity. The most promising radical scavenger currently being evaluated, is dexrazoxane.³⁷⁸ For doxorubicin treatment dexrazoxane is already recommended in disseminated disease after the cumulative dose of doxorubicin reaches 300 mg/m².¹⁹⁸ However, there is still no evidence for or against the use of dexrazoxane in the adjuvant setting for any tumor type. Because dexrazoxane theoretically may reduce the efficacy of anthracyclines, and because data are not yet available on long-term toxicity, further studies should be performed before this drug is used in the adjuvant setting. Although various risk-factors of anthracycline cardiotoxicity have been identified (e.g. a high cumulative dose, age > 70 years, pre-existent cardiac disease and a high peak dose), the wide variation in individual susceptibility of this side effect is still largely unexplained. It remains a challenge for future research to search for specific biochemical, molecular, or even genetic markers to predict an individual patient's susceptibility for anthracycline cardiotoxicity prior to the administration of chemotherapy. This would probably allow higher dosages of an-

thracyclines and thus a more effective tumor treatment in the patients who are less susceptible, and it might prevent the cardiotoxicity in the susceptible patients.

Although the aforementioned future developments may be able to prevent the cardiotoxicity, currently many patients are still be confronted with the potential risk of cardiotoxicity of anthracyclines. Therefore detection of cardiotoxicity before the onset of congestive heart failure is still important. The determination of the left ventricular ejection fraction remains, despite its limitations, the technique of choice in routine practice because no other technique has been shown to be a better alternative. However, further attempts should be made in the search for a more sensitive technique that is capable to detect cardiotoxicity in an early stage and that is suitable for routine use. Particularly biochemical markers of cardiac damage such as the natriuretic peptides,³⁶⁸ and possibly also cardiac troponine T³⁷⁹ deserve further evaluation, considering the ease of such test in routine practice. But further evaluation of sensitivity and specificity of these markers remains essential before they can be recommended for routine use. Possibly the recent recognition that apoptosis is also involved in the pathogenesis of anthracycline cardiotoxicity,³⁸⁰ this might lead to new detection methods such as in vivo visualization of apoptosis by technetium-99m-labelled annexin.³⁸¹

Furthermore, randomized trials are needed to investigate whether in patients with subclinical anthracycline cardiotoxicity, further deterioration of the cardiac function can be prevented by medical intervention with for example angiotensin converting enzyme inhibitors.

Since the efficacy of chemotherapy in breast cancer is still not optimized, new chemotherapy combinations are being explored, including the combination of anthracyclines with taxanes and the combination of anthracyclines with the monoclonal antibody Trastuzumab (Herceptin). Both combinations seem to be very effective in the treatment of breast cancer, but alarming reports have already appeared concerning possible enhancement of cardiotoxicity.^{382,383} Attention for cardiotoxicity will therefore remain an important issue in the expanding treatment of breast cancer.

CHAPTER 11

SUMMARY

As a consequence of the successful use of chemotherapy in the treatment of curable neoplasms such as testicular cancer, and the increasing application of (neo)adjuvant chemotherapy for various tumor types, including breast cancer, the number of patients with a presumed normal life expectancy after treatment is rising. Attention for long-term side effects is therefore of growing importance. Evaluation of chronic cardiovascular toxicity is of particular relevance since it may negatively influence the life expectancy after chemotherapy. A number of cytostatic agents is well-known for their toxic effects to the myocardium. Among these the most important are the anthracycline antibiotics, such as doxorubicin and its analogue epirubicin, and related compounds such as mitoxantrone. By far the most important aspect of cardiotoxicity induced by these agents is congestive heart failure, resulting from a cumulative dose-related myocardial damage. This damage occurs during chemotherapy, but it may take a long time before congestive heart failure develops. By means of non-invasive detection techniques myocardial damage can be determined in an early stage before congestive heart failure develops which may allow early medical intervention with the goal to prevent further progression. Until recently, other cytostatic agents such as used in the treatment of patients with disseminated testicular cancer, were not known to cause severe chronic cardiovascular toxicity. However, several reports have indicated that a part of the patients who survives disseminated testicular cancer develops an unfavorable cardiovascular risk-profile and therefore may be prone to develop cardiovascular disease.

The aim of this thesis as formulated in the introduction in **chapter 1** was to investigate the long-term effects of chemotherapy on the cardiovascular system, which was mainly studied in testicular cancer and breast cancer patients, treated at the University Hospital Groningen, the Netherlands.

Chapter 2 is a review of the literature on cardiovascular toxicity related to the use of cytostatic agents. Four patient groups with a good life expectancy after successful chemotherapy were selected to review this issue: survivors of metastatic testicular cancer and of malignant lymphomas treated with curative chemotherapy, and breast- and colon cancer patients treated with adjuvant chemotherapy. Besides direct toxic effects of chemotherapy on the cardiovascular system also the indirect toxic effects such as chemotherapy related metabolic and hormonal imbalances that may aggravate cardiovascular damage, are discussed. In survivors of testicular cancer the most evident type of long-term vascular toxicity is Raynaud's phenomenon. Furthermore, a part of this patient group develops an unfavorable cardiovascular risk-profile consisting of hypercholesterolemia and hypertension, which to our experience results in an increased risk of coronary artery disease. In survivors of malignant lymphomas and in patients treated with adjuvant chemotherapy for breast cancer the main type of cardiotoxicity is related to the use of anthracyclines, which can result in congestive heart failure. This risk of cardiotoxicity may be aggravated by the use of thoracic irradiation. In patients treated with adjuvant chemotherapy for colorectal cancer, only acute cardiotoxicity (mainly angina pectoris) is reported related to the use of 5-fluorouracil but this does not appear to be detrimental in the long-term.

In **Chapter 3** a review of the literature is presented on the detection of anthracycline-induced cardiotoxicity. Although endomyocardial biopsy is considered to be

the most sensitive and specific test for this purpose, its use is limited by its invasiveness. In daily practice oncologists usually make use of the non-invasive determination of the left ventricular ejection fraction to detect early cardiotoxicity, but this method is not suitable to identify cardiotoxicity in an early stage. Based on increasing knowledge into the pathophysiology of anthracycline-induced cardiotoxicity and heart failure in general, new methods including the determination of diastolic function parameters, heart rate variability, and natriuretic peptides have been proposed to identify patients at risk for the development of congestive heart failure in an early stage. However, most of these newer methods have not yet been adequately evaluated to allow them to be recommended for use in routine clinical practice.

In **Chapter 4** the results are presented of a follow-up study in testicular cancer survivors who had been treated 10 to 20 years previously with cisplatin based chemotherapy. Since in previous studies it was shown that a number of testicular cancer patients develops an unfavorable cardiovascular risk-profile after chemotherapy, consisting of hypercholesterolemia, overweight and hypertension, we investigated whether these cured patients in the long-term, are at an increased risk of developing cardiovascular morbidity and mortality. Therefore 87 patients treated with cisplatin containing chemotherapy who were still in remission for at least 10 years and whose age was 50 years or less at the time of the analysis, were evaluated for the occurrence of cardiovascular events. Major cardiac events were found in 5 of the 87 patients (age at time of event 30-42 years; 9-16 years after chemotherapy): 2 patients had a myocardial infarction and 3 patients angina pectoris with proven myocardial ischemia. An increased risk for coronary artery disease, as compared to the general male Dutch population was found. Of these 87 patients, 62 were additionally evaluated for cardiac damage and cardiovascular risk factors. Their cardiovascular risk profile was compared to that of 40 patients with comparable age and follow-up duration treated with orchidectomy only for stage I testicular cancer. Echocardiography in the patients treated with chemotherapy showed a normal systolic left ventricular function in most patients, but the diastolic left ventricular function was disturbed in 33%. Moreover, 79% had hypercholesterolemia, 39% hypertension, 25% still experienced Raynaud's phenomenon, and 22 % had microalbuminuria probably reflecting endothelial dysfunction. Compared with stage I patients, the chemotherapy treated patients had higher blood pressures, higher total serum cholesterol- and triglyceride levels and were more insulin resistant. The pathogenesis of the observed increased risk of coronary artery disease is unclear, but this may be linked with several factors, including metabolic and hormonal changes related to gonadal toxicity, possibly resulting in the development of a syndrome X- like state. The latter is suggested by the combination of overweight, microalbuminuria, hypertension, insulin resistance and dyslipidemia found in some of these patients. Furthermore, the induction of chronic endothelial dysfunction may play a role. Accurate follow-up, focused on cardiovascular complications and aiming at intervention in these young cancer-survivors, appears to be of great importance in the near future.

Exposure to cisplatin combination chemotherapy seems with the current data an important contributing factor in the occurrence of cardiovascular disease in long-term survivors of metastatic testicular cancer. The interval between chemotherapy treatment in these patients and the development of cardiovascular disease may take

more than 10 years.

For this reason we hypothesized in **chapter 5** whether the observed increased risk of cardiovascular disease might be related to prolonged retention of cisplatin in the body. Therefore we measured plasma platinum concentrations using a very sensitive assay. In this assay high-pressure decomposition of samples was performed, followed by an adsorptive voltammetric measurement. Plasma platinum concentrations in the patients studied were compared with those of 20 control patients who were cured from stage I testicular cancer by orchidectomy without chemotherapy. In all 61 tested patients who were 10-20 years after cisplatin administration, platinum was detectable with a mean concentration of 64.9 pg/g plasma, whereas in the control group platinum concentrations were not detectable. The height of the platinum concentration was related to the cumulative dose administered and inversely related to the renal function. These results suggest that platinum is recirculating in the plasma between potential body stores such as the liver, bone, and muscle. These chronic low plasma concentrations of platinum may play a role in the development of cardiovascular morbidity after chemotherapy.

In **chapter 6** we describe the results of the investigations regarding the magnesium status in long-term survivors of testicular cancer. Hypomagnesemia in general has been associated with hypertension, abnormal lipid metabolism, and accelerated atherosclerosis, and the occurrence of vascular ischemic events. Cisplatin can cause proximal-tubule damage that results in renal magnesium wasting. The time during which this magnesium depletion persists after treatment is not known. Since about 99 % of the total body magnesium is located intracellularly, determination of the red-blood cell magnesium concentration might be a better indicator of the magnesium status than the serum magnesium concentration. In addition, a low intracellular magnesium content is associated with hypertension and insulin-resistance. Both red-blood cell magnesium concentration (assessed by an indirect method) and the serum magnesium concentration were measured in 50 survivors of testicular cancer who were 10 to 20 years after treatment. These data were compared with the magnesium status of a control group of 17 patients with testicular cancer not treated with chemotherapy. We found that although the mean serum concentration of magnesium in both groups did not differ, the red-blood cell magnesium content in the group of patients treated with chemotherapy was significantly lower than in the control group. These data suggest that up to 20 years after cisplatin-containing chemotherapy survivors of testicular cancer are still magnesium deficient, although the serum magnesium concentration is normal. This disturbed magnesium equilibrium might play a role in the development of unfavorable cardiovascular risk-profile observed in these patients.

In **chapter 7** we describe a (pilot) study performed in breast cancer patients, who had been treated with anthracyclines and irradiation, in order to investigate early signs of cardiotoxicity in these patients. These patients had all participated in high-dose chemotherapy schedules including autologous bone- or stem cell transplantation. We tested whether analysis of the heart rate variability calculated from a 24-hr Holter registration, as measure for the autonomic tone, and assessment of the diastolic function by echocardiography could help to identify early signs of cardiotoxicity. Therefore twenty females treated for breast cancer with high-dose anthracycline-

based chemotherapy were evaluated. None of these patients had signs or symptoms of heart disease and all had a normal systolic left ventricular function. One or more echocardiographic diastolic parameters were abnormal in 50 % of the patients. HRV frequency parameters were even abnormal in 85 % of the patients. Mean values of both time domain and frequency domain parameters were decreased, in particular the parasympathetic indexes compared to a control group consisting of healthy age-matched females. It was concluded that autonomic impairment occurs in a large proportion of asymptomatic patients with normal systolic LV function after high-dose anthracycline-based chemotherapy and seems to be an early sign of cardiotoxicity after treatment.

To explore these findings further in a more homogenous population we evaluated in **chapter 8** in a cross-sectional design the cardiac function by means of various detection techniques in 56 breast cancer patients two or more years after epirubicin-containing chemotherapy combined with locoregional irradiation. Patients had been treated within a randomized phase III study comparing 5 cycles of 5-fluorouracil, epirubicin (90 mg/m^2), and cyclophosphamide (FEC) ($n=30$) with 4 cycles of FEC followed by high-dose chemotherapy consisting of cyclophosphamide (6g/m^2), thiotepa and carboplatin ($n=26$). The main findings of this study showed that 11% of all patients had a decreased systolic function and 38% an impaired diastolic function. HRV parameters reflecting vagal activity in the whole patient group were significantly impaired compared to healthy age-matched controls. In 30% of the patients exertional dyspnea was present. These patients were characterized by an impaired diastolic function and a reduced HRV compared to patients without these symptoms. No difference in cardiotoxicity was found between the treatment regimens. This study showed that in a substantial number of breast cancer patients treated with a relatively low cumulative dose of epirubicin signs of mild chronic cardiotoxicity are present two or more (maximum 6.5) years after treatment.

Following this cross-sectional study, we describe in **chapter 9** the results of a prospective evaluation of the cardiac function of 40 breast cancer patients who were treated in the same phase III trial as described in chapter 8. Of them 21 patients were treated with 5 cycles of FEC and 19 with 4 cycles of FEC followed by high-dose chemotherapy consisting of cyclophosphamide, thiotepa and carboplatin. The cardiac function of these patients was evaluated before chemotherapy, after completion of chemotherapy but before radiotherapy, after radiotherapy and one year after chemotherapy. During the studied period, none of the patients in this study developed congestive heart failure. However, various indications of an impairment of the cardiac function were observed including a decline in the mean left ventricular ejection fraction (LVEF), resulting in an abnormal LVEF in 17% of the patients. Plasma levels of natriuretic peptides increased gradually in the course of time and an increase in the QTc time was observed. No persistent alterations were found in the diastolic function and the HRV, although the HRV was temporarily reduced shortly after chemotherapy in patients treated with the high-dose regimen. The observed changes in cardiac function indicate mild cardiotoxicity one year after start of treatment which in some of these patients may result in an increased risk for development of late-onset congestive heart failure.

In **chapter 10** suggestions with respect to further research are described. It is important to outline the prevalence of cardiovascular toxicity due to cytostatic agents and to investigate the pathogenesis. Thereafter attempts should be made to ameliorate or even prevent the cardiovascular side effects of cytostatic agents. The ultimate goal will be adding to a successful treatment of patients with cancer, a maximization of their chances of a long and healthy life by designing strategies for primary and secondary prevention of long-term toxic effects of chemotherapy.

NEDERLANDSE SAMENVATTING

Door het succes van chemotherapie in de behandeling van patiënten met uitzaaiingen van testiskanker, en het toenemende gebruik van (neo)adjuvante chemotherapie voor verschillende andere soorten kanker, waaronder borstkanker, is het aantal patiënten met een veronderstelde normale levensverwachting na behandeling aan het toenemen. Daarom wordt de aandacht voor lange termijn effecten van de chemotherapie in toenemende mate van belang. Met name is onderzoek naar het vóórkomen van chronische cardiovasculaire toxiciteit van belang omdat deze vorm van toxiciteit de kwaliteit van leven en de levensverwachting na chemotherapie negatief kan beïnvloeden. Van een aantal cytostatica is reeds lange tijd bekend dat zij schade aan het myocard (hartspierweefsel) kunnen geven. De belangrijkste hiervan zijn de anthracycline antibiotica, zoals doxorubicine en epirubicine, en hieraan gerelateerde cytostatica zoals mitoxantrone. De klinisch meest belangrijke vorm van cardiotoxiciteit die wordt veroorzaakt door anthracyclines is hartfalen, dat het gevolg is van myocard schade. Het optreden van deze schade hangt samen met de cumulatieve dosis van de toegevoegde anthracyclines. Deze myocard schade ontstaat reeds tijdens de chemotherapie, maar het kan lang duren voordat er klachten en verschijnselen ontstaan. Met behulp van non-invasieve detectiemethoden kan de myocard schade in een vroeg stadium, voordat het hartfalen zich manifesteert, worden opgespoord. Dit maakt misschien vroegtijdige behandeling met medicijnen mogelijk, met als doel verdere verslechtering van de hartfunctie te voorkomen.

Tot voor kort was het van andere cytostatica zoals die bijvoorbeeld gebruikt worden bij de behandeling van patiënten met uitgezaaide testiskanker, niet goed bekend of deze ook op de lange termijn chronische cardiovasculaire toxiciteit zouden kunnen veroorzaken. Wel was uit vroegere onderzoeken gebleken dat een aantal van deze patiënten na behandeling met chemotherapie, een ongunstig cardiovasculair risicoprofiel ontwikkelt, hetgeen zou kunnen leiden tot een grotere kans op het ontstaan van hart- en vaatziekten.

Het doel van dit proefschrift, zoals beschreven in **hoofdstuk 1**, is de lange termijn effecten van chemotherapie op het cardiovasculaire systeem te onderzoeken. Dit onderzoek vond plaats bij patiënten met testiskanker en bij patiënten met borstkanker, die allen behandeld werden in het Academisch Ziekenhuis Groningen, Nederland.

Hoofdstuk 2 geeft een overzicht van de literatuur betreffende cardiovasculaire toxiciteit, samenhangend met het gebruik van cytostatica. Vier groepen patiënten met een goede levensverwachting na chemotherapie werden uitgezocht om dit onderwerp te bespreken: genezen patiënten met uitgezaaide testiskanker of lymfeklierkanker, en patiënten met borst- of darmkanker die behandeld waren met adjuvante chemotherapie. Naast de directe toxische effecten van chemotherapie op het cardiovasculaire systeem, werden ook de indirecte toxische effecten besproken, zoals de door chemotherapie veroorzaakte metabole en hormonale veranderingen die de kans op cardiovasculaire ziekten kunnen vergroten. Bij patiënten die waren genezen van uitgezaaide testiskanker is de meest voorkomende vorm van chronische vasculaire schade het fenomeen van Raynaud. Verder ontstaat bij een aantal patiënten een ongunstig cardiovasculair risicoprofiel dat volgens onze eigen bevindingen leidt tot een

verhoogde kans op cardiovasculaire ziekte.

Bij patiënten die genezen zijn van hun lymfeklierkanker en bij patiënten die met adjuvante chemotherapie worden behandeld voor borstkanker, wordt het belangrijkste deel van de cardiotoxiciteit veroorzaakt door het gebruik van de anthracyclines, dat kan resulteren in klinisch hartfalen. De kans dat deze cardiotoxiciteit ontstaat zou vergroot kunnen worden door gelijktijdige of latere bestraling van de borstkas.

Hoofdstuk 3 geeft een overzicht van de literatuur betreffende de detectietechnieken van de door anthracycline veroorzaakte cardiotoxiciteit. Hoewel een hartspierbiopsie wordt beschouwd als de meest gevoelige en specifieke test, is de toepassing van deze methode ongeschikt voor routine gebruik. In de dagelijkse praktijk wordt meestal gebruik gemaakt van de non-invasieve bepaling van de uitdrijfkraft van de linker kamer, de zgn. ejectie fractie van de linker ventrikel. Deze methode is echter ongeschikt om in een vroeg stadium cardiotoxiciteit vast te stellen. Gebaseerd op een toenemend inzicht in de pathofysiologie van anthracycline-veroorzaakte cardiotoxiciteit en van hartfalen in het algemeen, zijn nieuwere technieken voorgesteld zoals de bepaling van diastolische functie parameters, de analyse van de heart rate variability, en de bepaling van natriuretische peptiden in het bloed met als doel patiënten met een verhoogd risico op hartfalen in een vroeg stadium op te sporen.

In **hoofdstuk 4** worden de resultaten gepresenteerd van een vervolgstudie bij patiënten met testiskanker. Deze patiënten werden 10 tot 20 jaar geleden behandeld met cisplatine bevattende chemotherapie. Omdat uit eerdere studies was gebleken dat een aantal patiënten een ongunstig cardiovasculair risicoprofiel ontwikkelt, bestaande uit hypercholesterolemie, hypertensie en overgewicht, hebben we onderzocht of deze patiënten ook een verhoogd risico hebben op het ontstaan van cardiovasculaire ziekte en sterfte. Daartoe werden 87 jonge patiënten die na behandeling met cisplatine bevattende chemotherapie tenminste 10 jaar in blijvende remissie waren, onderzocht op het voorkomen van cardiovasculaire ziekten. Cardiale afwijkingen werden bij 5 van deze 87 patiënten gevonden (2 patiënten kregen een myocardiinfarct en 3 patiënten hadden angina pectoris met bewezen myocard ischemie). In vergelijking met de normale Nederlandse bevolking werd een verhoogd risico op het ontstaan van coronair lijden gevonden.

Bij 62 van deze patiënten werd vervolgens aanvullend onderzoek verricht. Hierbij werd het cardiovasculaire risicoprofiel vergeleken met dat van 40 patiënten van vergelijkbare leeftijd en follow-up duur, waarbij alleen de testis was verwijderd. Vergeleken met de patiënten waarbij alleen de testis was verwijderd, hadden de patiënten die behandeld waren met chemotherapie, een hogere bloeddruk, hogere cholesterol en triglyceride spiegels in het bloed en bleken ze minder gevoelig te zijn voor insuline.

Onderzoek naar de functie van het hart door middel van geluidsonderzoek (echocardiografie) liet zien dat bij de patiënten die behandeld waren met chemotherapie de uitdrijffunctie van de linker kamer meestal normaal was, maar dat de diastolische functie gestoord was bij 33% van de patiënten. Bovendien had 25% van de patiënten nog steeds last van het fenomeen van Raynaud, en 22% van de patiënten had microalbuminurie hetgeen waarschijnlijk een uiting is van endotheel disfunctie.

De oorzaak van het verhoogde risico op het ontstaan van coronair lijden na cis-

platine bevattende chemotherapie is onduidelijk, maar het zou te maken kunnen hebben met verschillende factoren, zoals metabole en hormonale veranderingen ten gevolge van de gonadale toxiciteit van de chemotherapie. Deze toxiciteit leidt tot een 'syndroom X'-achtig beeld bestaand uit een combinatie van overgewicht, microalbuminurie hypertensie, insuline resistentie en dislipidemie. Verder zou het veroorzaken van chronische endotheelschade ook een rol kunnen spelen. Nauwkeurig vervolg onderzoek bij deze patiënten is van groot belang. De aandacht moet hierbij niet alleen uitgaan naar het optreden van cardiovasculaire complicaties, maar ook naar mogelijke preventieve en therapeutische interventies.

Blootstelling aan cisplatine combinatie chemotherapie lijkt met deze data een belangrijke bijdragende factor in het optreden van cardiovasculaire ziekte in patiënten genezen van uitzaaiingen van testiskanker. Het interval tussen de chemotherapie en het optreden van cardiovasculaire ziekte kan langer dan 10 jaar duren.

Daarom veronderstelden we in **hoofdstuk 5** dat het verhoogde risico op cardiovasculaire ziekte na behandeling met cisplatine combinatie chemotherapie in relatie zou kunnen staan tot langdurige aanwezigheid van cisplatine het lichaam. Daartoe werden platinum concentraties in het plasma gemeten met een gevoelige assay. De plasma platinum concentraties van behandelde patiënten werden vergeleken met die van 20 controle patiënten waarbij alleen een testis was verwijderd. In het plasma van alle 61 onderzochte patiënten was platinum detecteerbaar (gemiddelde concentratie van 64.9 pg/g plasma) ondanks het feit dat deze patiënten 10-20 jaar eerder met cisplatine bevattend chemotherapie waren behandeld. In de controle groep was platinum niet in het plasma detecteerbaar. De hoogte van de platinum concentratie bij de behandelde patiënten was positief gecorreleerd met de toegediende cumulatieve dosering cisplatine en negatief gecorreleerd met de nierfunctie. Uit deze resultaten blijkt dat platinum waarschijnlijk recirculeert tussen verschillende lichaams depots zoals de lever, beenmerg en spieren. Deze chronische lage platinum concentraties in het plasma zouden een rol kunnen spelen in de ontwikkeling van cardiovasculaire ziekte na chemotherapie.

In **hoofdstuk 6** beschrijven we de resultaten van een onderzoek naar het magnesium evenwicht bij patiënten die genezen zijn van testiskanker. Een te laag magnesium gehalte in het bloed heeft een mogelijke relatie met het ontstaan van hypertensie, een afwijkend lipidenprofiel, en een versnelde atherosclerose. Cisplatine veroorzaakt schade aan de proximale tubuli in de nieren dat resulteert in verlies van magnesium via de urine. Hoe lang dit magnesium verlies aanwezig blijft na de chemotherapie is niet precies bekend. Omdat ongeveer 99% van het totale lichaams magnesium zich intracellulair bevindt, zou het bepalen van de magnesium concentratie in de rode bloedlichaampjes een betere maat kunnen zijn van het magnesiumevenwicht dan de bepaling van het magnesium in het serum. Bovendien, zou een lage magnesium concentratie in de rode bloedlichaampjes in verband staan met hypertensie en insuline resistentie. Zowel de concentratie van magnesium in de rode bloedlichaampjes als in het serum werd gemeten bij 50 patiënten, die 10 tot 20 jaar eerder met chemotherapie waren behandeld. De gegevens werden vergeleken met die van een controle groep bestaande uit 17 testiskanker patiënten waar de behandeling alleen bestond uit verwijdering van de testis. We vonden dat geen verschil bestond in de

gemiddelde serum magnesium concentratie, maar wel in de intracellulaire magnesium concentratie. Deze was namelijk in de chemotherapie groep lager dan in de controle groep. Deze gegevens suggereren dat tot 20 jaar na een behandeling met cisplatine-bevattende chemotherapie, patiënten nog steeds een magnesium tekort hebben ondanks het feit dat de magnesium concentratie in het serum normaal is. Dit verstoorde magnesium evenwicht zou een rol kunnen spelen in de ontwikkeling van het ongunstige cardiovasculaire risicoprofiel dat in deze patiënten wordt gevonden.

In **hoofdstuk 7** beschrijven we een oriënterend onderzoek naar het vóórkomen van vroege tekenen van cardiotoxiciteit. Dit onderzoek is uitgevoerd bij borstkanker patiënten die behandeld waren met anthracyclines als onderdeel van hoog gedoseerde chemotherapie en autologe stamcel- of beenmerg transplantatie en bestraling. We onderzochten of heart rate variability (HRV) analyse berekend op basis van een 24 uren ECG-registratie, en het bepalen van de diastolische functie van het hart door middel van echocardiografie van waarde zou kunnen zijn. Deze onderzoeken werden verricht bij 20 vrouwen die behandeld waren voor borstkanker. Niemand was tevoren bekend met een hartziekte en alle patiënten hadden een normale systolische linker ventrikel functie. We vonden dat één of meerdere diastolische parameters afwijkend waren in 50% van de patiënten. De HRV frequentie domein parameters bleken zelfs afwijkend te zijn bij 85% van de patiënten. De gemiddelde waarden van zowel de tijd domein als ook de frequentie domein parameters waren verlaagd. Wanneer deze gegevens werden vergeleken met die van een controlegroep bestaande uit gezonde leeftijdsgenoten, dan bleken vooral de parasympatische parameters te zijn verlaagd. We concludeerden dat een verslechtering van de autonome functie voor komt bij een groot deel van nog asymptomatische patiënten met een normale systolische linker ventrikel functie na anthracycline behandeling. Deze verslechtering lijkt een vroeg teken van cardiotoxiciteit te zijn, hoewel de linker ventrikel nog een normale uitdrijf functie heeft.

In **hoofdstuk 8** wordt een eenmalig onderzoek naar de cardiale functie van 56 patiënten twee of meer jaren na epirubicine-bevattende chemotherapie gecombineerd met locoregionale radiotherapie beschreven. Patiënten waren behandeld in het kader van een gerandomiseerde studie waarin de effectiviteit van 5 kuren van 5-fluorouracil, epirubicine en cyclophosphamide (FEC) ($n=30$) werd vergeleken met die van 4 kuren FEC gevolgd door hoge dosis chemotherapie bestaande uit cyclophosphamide, thiotepa, en carboplatin ($n=26$). De belangrijkste uitkomsten van dit onderzoek waren dat 11% van de patiënten een verminderde systolische en 38% een verminderde diastolische hartfunctie had. Ook de HRV parameters die de parasympatische activiteit weerspiegelen, waren verlaagd. Dertig procent van de patiënten klaagde over kortademigheid bij inspanning. Deze patiënten bleken een verminderde diastolische functie en een lagere HRV te hebben vergeleken met patiënten zonder deze klachten. Er kon geen verschil aangetoond worden in het vóórkomen van cardiotoxiciteit tussen de beide behandeling regimes. Deze studie liet zien dat bij een aanzienlijk deel van de patiënten behandeld met relatief lage cumulatieve dosis ($360\text{--}450\text{mg/m}^2$) epirubicine tekenen van geringe cardiotoxiciteit aanwezig zijn.

In **hoofdstuk 9** beschrijven we de resultaten van een prospectieve herhaalde evaluatie van de cardiale functie van 40 borstkanker patiënten. Ook deze patiënten

werden behandeld in dezelfde gerandomiseerde studie als beschreven in hoofdstuk 8. In totaal werden 21 patiënten behandeld met 5 FEC kuren en 19 patiënten met 4 FEC kuren gevolg door hoge-dosis chemotherapie. De cardiale functie van deze patiënten werd gemeten voor het begin van de chemotherapie, na beëindiging van chemotherapie maar voor het begin van de radiotherapie, na radiotherapie en een jaar na chemotherapie. Geen van deze patiënten ontwikkelde klinisch verschijnselen van hartfalen. Er werden echter wel verschillende aanwijzingen gevonden voor een verslechtering van de hartfunctie, zoals een afname van de gemiddelde linker ventrikel ejectiefractie (LVEF), hetgeen resulteerde in een abnormale LVEF bij 17 % van de patiënten. Verder namen de plasma spiegels van natriuretische peptiden in de loop van de tijd geleidelijk toe en werd er een toename van de QTc tijd vastgesteld. Blijvende veranderingen werden niet gevonden in de diastolische functie en de HRV, hoewel een tijdelijke vermindering in HRV werd gemeten bij patiënten die behandeld waren met de hoge-dosis chemotherapie. De vastgestelde veranderingen in de cardiale functie tonen dat 1 jaar na het begin van de chemotherapie er nog sprake is van geringe cardiotoxiciteit die bij sommige patiënten wellicht kan leiden tot een verhoogd risico op het ontwikkelen van klinisch hartfalen.

In **hoofdstuk 10** worden suggesties voor verder onderzoek gegeven. Het is van belang het vóórkomen van chronische cardiovasculaire toxiciteit van cytostatica vast te stellen en de pathogenese te onderzoeken. Vervolgens moeten er pogingen worden gedaan om deze cardiovasculaire schade te verminderen of zelfs te voorkomen. Het uiteindelijke doel is om bij patiënten met kanker naast een succesvolle chemotherapie behandeling, de kans op een lang en gezond leven na chemotherapie zo groot mogelijk te maken, door het bedenken van strategieën om schadelijke lange termijn effecten te voorkomen.

REFERENCES

- 1 Shan K, Lincoff AM, Young JB. Anthracycline-induced cardiotoxicity. *Ann Intern Med* 1996;125:47-58.
- 2 Von Hoff DD, Layard MW, Basa P, Davis HL, Jr., Von Hoff AL, Rozencweig M et al. Risk factors for doxorubicin-induced congestive heart failure. *Ann Intern Med* 1979;91:710-717.
- 3 Steinherz LJ, Steinherz PG, Tan CT, Heller G, Murphy ML. Cardiac toxicity 4 to 20 years after completing anthracycline therapy. *JAMA* 1991;266:1672-1677.
- 4 Steinherz LJ, Steinherz PG, Tan C. Cardiac failure and dysrhythmias 6-19 years after anthracycline therapy: a series of 15 patients. *Med Pediatr Oncol* 1995;24:352-361.
- 5 Lipshultz SE, Lipsitz SR, Mone SM, Goorin AM, Sallan SE, Sanders SP et al. Female sex and drug dose as risk factors for late cardiotoxic effects of doxorubicin therapy for childhood cancer. *N Engl J Med* 1995;332:1738-1743.
- 6 International Germ Cell Consensus Classification: a prognostic factor- based staging system for metastatic germ cell cancers. International Germ Cell Cancer Collaborative Group. *J Clin Oncol* 1997;15:594-603.
- 7 Boyer M, Raghavan D. Toxicity of treatment of germ cell tumors. *Semin Oncol* 1992;19:128-142.
- 8 Hartmann JT, Kollmannsberger C, Kanz L, Bokemeyer C. Platinum organ toxicity and possible prevention in patients with testicular cancer. *Int J Cancer* 1999;83:866-869.
- 9 Vogelzang NJ, Bosl GJ, Johnson K, Kennedy BJ. Raynaud's phenomenon: a common toxicity after combination chemotherapy for testicular cancer. *Ann Intern Med* 1981;95:288-292.
- 10 Doll DC, List AF, Greco FA, Hainsworth JD, Hande KR, Johnson DH. Acute vascular ischemic events after cisplatin-based combination chemotherapy for germ-cell tumors of the testis. *Ann Intern Med* 1986;105:48-51.
- 11 Stefanelli T, Kuzmits R, Ulrich W, Glogar D. Acute vascular toxicity after combination chemotherapy with cisplatin, vinblastine, and bleomycin for testicular cancer. *Eur Heart J* 1988;9:552-556.
- 12 Icli F, Karaoguz H, Dincel D, Demirkazik A, Gunel N, Karaoguz R et al. Severe vascular toxicity associated with cisplatin-based chemotherapy. *Cancer* 1993;72:587-593.
- 13 Nichols CR, Roth BJ, Williams SD, Gill I, Muggia FM, Stablein DM et al. No evidence of acute cardiovascular complications of chemotherapy for testicular cancer: an analysis of the Testicular Cancer Intergroup Study. *J Clin Oncol* 1992;10:760-765.
- 14 Bokemeyer C, Berger CC, Kuczyk MA, Schmoll HJ. Evaluation of long-term toxicity after chemotherapy for testicular cancer. *J Clin Oncol* 1996;14:2923-2932.
- 15 Gietema JA, Sleijfer DTh, Willemse PHB, Schraffordt Koops H, van Ittersum E, Verschuren WM et al. Long-term follow-up of cardiovascular risk factors in patients given chemotherapy for disseminated nonseminomatous testicular cancer. *Ann Intern Med* 1992;116:709-715.
- 16 Raghavan D, Cox K, Childs A, Crygier J, Sullivan D. Hypercholesterolemia after chemotherapy for testis cancer. *J Clin Oncol* 1992;10:1386-1389.
- 17 Misset JL, di Palma M, Delgado M, Plagne R, Chollet P, Fumoleau P et al. Adjuvant treatment of node-positive breast cancer with cyclophosphamide, doxorubicin, fluorouracil, and vincristine versus cyclophosphamide, methotrexate, and fluorouracil: final report after a 16-year median follow-up duration. *J Clin Oncol* 1996;14:1136-1145.
- 18 Levine MN, Bramwell VH, Pritchard KI, Norris BD, Shepherd LE, Abu-Zahra H et al. Randomized trial of intensive cyclophosphamide, epirubicin, and fluorouracil chemotherapy compared with cyclophosphamide, methotrexate, and fluorouracil in premenopausal women with node-positive breast cancer. National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 1998;16:2651-2658.
- 19 Cohn JN. Prevention of heart failure. *Cardiology* 1999;92 Suppl 1:22-25.
- 20 McKelvie RS, Benedict CR, Yusuf S. Evidence based cardiology: prevention of congestive heart failure and management of asymptomatic left ventricular dysfunction. *BMJ* 1999;318:1400-1402.
- 21 McKillop JH, Bristow MR, Goris ML, Billingham ME, Bockemuehl K. Sensitivity and specificity of radionuclide ejection fractions in doxorubicin cardiotoxicity. *Am Heart J* 1983;106:1048-1056.
- 22 Stoddard MF, Seeger J, Liddell NE, Hadley TJ, Sullivan DM, Kupersmith J. Prolongation of isovolumetric relaxation time as assessed by Doppler echocardiography predicts doxorubicin-induced systolic dysfunction in humans. *J Am Coll Cardiol* 1992;20:62-69.
- 23 Schmitt K, Tulzer G, Merl M, Aichhorn G, Grillenberger A, Wiesinger G et al. Early detection of doxorubicin and daunorubicin cardiotoxicity by echocardiography: diastolic versus systolic parameters. *Eur J Pediatr* 1995;154:201-204.
- 24 Postma A, Bink-Boelkens MT, Beaufort-Krol GC, Kengen RA, Elzenga NJ, Schasfoort-van Leeuwen MJ et al. Late cardiotoxicity after treatment for a malignant bone tumor. *Med Pediatr Oncol* 1996;26:230-237.

- 25 Tothill P, Klys HS, Matheson LM, McKay K, Smyth JF. The long-term retention of platinum in human tissues following the administration of cisplatin or carboplatin for cancer chemotherapy. *Eur J Cancer* 1992;28A:1358-1361.
- 26 Schierl R, Rohrer B, Hohnloser J. Long-term platinum excretion in patients treated with cisplatin. *Cancer Chemother Pharmacol* 1995;36:75-78.
- 27 Luthringer C, Rayssiguier Y, Gueux E, Berthelot A. Effect of moderate magnesium deficiency on serum lipids, blood pressure and cardiovascular reactivity in normotensive rats. *Br J Nutr* 1988;59:243-250.
- 28 Altura BM, Altura BT, Gebrewold A, Ising H, Gunther T. Magnesium deficiency and hypertension: correlation between magnesium-deficient diets and microcirculatory changes in situ. *Science* 1984;223:1315-1317.
- 29 Turlapaty PD, Altura BM. Magnesium deficiency produces spasms of coronary arteries: relationship to etiology of sudden death ischemic heart disease. *Science* 1980;208:198-200.
- 30 Schilsky RL, Anderson T. Hypomagnesemia and renal magnesium wasting in patients receiving cisplatin. *Ann Intern Med* 1979;90:929-931.
- 31 Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. *N Engl J Med* 1998;339:900-905.
- 32 Becker K, Erckenbrecht JF, Haussinger D, Frieling T. Cardiotoxicity of the antiproliferative compound fluorouracil. *Drugs* 1999;57:475-484.
- 33 Lipshultz SE, Colan SD, Gelber RD, Perez-Atayde AR, Sallan SE, Sanders SP. Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukemia in childhood. *N Engl J Med* 1991;324:808-815.
- 34 Vokes EE. Combined modality therapy of solid tumours. *Lancet* 1997;349 Suppl 2:SII4-SII6.
- 35 Einhorn LH. Testicular cancer as a model for a curable neoplasm: The Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res* 1981;41:3275-3280.
- 36 Einhorn LH, Williams SD, Troner M, Birch R, Greco FA. The role of maintenance therapy in disseminated testicular cancer. *N Engl J Med* 1981;305:727-731.
- 37 Williams SD, Birch R, Einhorn LH, Irwin L, Greco FA, Loehrer PJ. Treatment of disseminated germ-cell tumors with cisplatin, bleomycin, and either vinblastine or etoposide. *N Engl J Med* 1987;316:1435-1440.
- 38 Bosl GJ, Motzer RJ. Testicular germ-cell cancer. *N Engl J Med* 1997;337:242-253.
- 39 Rodriguez J, Collazos J, Gallardo M, Hernando G. Angina pectoris following cisplatin, etoposide, and bleomycin in a patient with advanced testicular cancer. *Ann Pharmacother* 1995;29:138-139.
- 40 Samuels BL, Vogelzang NJ, Kennedy BJ. Severe vascular toxicity associated with vinblastine, bleomycin, and cisplatin chemotherapy. *Cancer Chemother Pharmacol* 1987;19:253-256.
- 41 Gerl A, Clemm C, Schleuning M, Wilmanns W. Fatal cerebrovascular accident associated with chemotherapy for testicular cancer [letter]. *Eur J Cancer* 1993;29A:1220-1221.
- 42 Lajer H, Daugaard G. Cisplatin and hypomagnesemia. *Cancer Treat Rev* 1999;25:47-58.
- 43 Lejonec JL, Vernant JP, Macquin J, Castaigne A. Myocardial infarction following vinblastine treatment [letter]. *Lancet* 1980;2:692.
- 44 Airey CL, Dodwell DJ, Joffe JK, Jones WG. Etoposide-related myocardial infarction. *Clin Oncol (R Coll Radiol)* 1995;7:135.
- 45 Czaykowski PM, Moore MJ, Tannock IF. High risk of vascular events in patients with urothelial transitional cell carcinoma treated with cisplatin based chemotherapy. *J Urol* 1998;160:2021-2024.
- 46 Cantwell BM, Mannix KA, Roberts JT, Ghani SE, Harris AL. Thromboembolic events during combination chemotherapy for germ cell malignancy [letter]. *Lancet* 1988;2:1086-1087.
- 47 Lederman GS, Garnick MB. Pulmonary emboli as a complication of germ cell cancer treatment. *J Urol* 1987;137:1236-1237.
- 48 Hall MR, Richards MA, Harper PG. Thromboembolic events during combination chemotherapy for germ cell malignancy [letter]. *Lancet* 1988;2:1259.
- 49 Jackson AM, Rose BD, Graff LG, Jacobs JB, Schwartz JH, Strauss GM et al. Thrombotic microangiopathy and renal failure associated with antineoplastic chemotherapy. *Ann Intern Med* 1984;101:41-44.
- 50 Licciardello JT, Moake JL, Rudy CK, Karp DD, Hong WK. Elevated plasma von Willebrand factor levels and arterial occlusive complications associated with cisplatin-based chemotherapy. *Oncology* 1985;42:296-300.
- 51 Subar M, Muggia FM. Apparent myocardial ischemia associated with vinblastine administration. *Cancer Treat Rep* 1986;70:690-691.
- 52 Illarramendi JJ, Gallego J. Cisplatin-based chemotherapy and acute cerebrovascular events [letter]. *Lancet* 1991;338:705.
- 53 Gerl A, Clemm C, Wilmanns W. Acute cerebrovascular event after cisplatin-based chemotherapy for testicular cancer [letter]. *Lancet* 1991;338:385-386.

- 54 Adamson IY, Bowden DH. The pathogenesis of bleomycin-induced pulmonary fibrosis in mice. *Am J Pathol* 1974;77:185-197.
- 55 Sleijfer S, van der Mark TW, Schraffordt Koops H, Mulder NH. Decrease in pulmonary function during bleomycin-containing combination chemotherapy for testicular cancer: not only a bleomycin effect. *Br J Cancer* 1995;71:120-123.
- 56 Van Barneveld PW, Sleijfer DTh, van der Mark TW, Mulder NH, Schraffordt Koops H, Sluiter HJ et al. Natural course of bleomycin-induced pneumonitis. A follow-up study. *Am Rev Respir Dis* 1987;135:48-51.
- 57 Doll DC, Ringenberg QS, Yarbrow JW. Vascular toxicity associated with antineoplastic agents. *J Clin Oncol* 1986;4:1405-1417.
- 58 Dirix LY, Libura M, Libura J, Vermeulen PB, De Bruijn EA, van Oosterom AT. In vitro toxicity studies with mitomycins and bleomycin on endothelial cells. *Anticancer Drugs* 1997;8:859-868.
- 59 Phan SH, Gharee-Kermani M, Wolber F, Ryan US. Bleomycin stimulates production of transforming growth factor-beta by rat pulmonary artery endothelial cells. *Chest* 1991;99:665.
- 60 Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage. The Steno hypothesis. *Diabetologia* 1989;32:219-226.
- 61 Pedrinelli R, Giampietro O, Carmassi F, Melillo E, Dell'Omo G, Catapano G et al. Microalbuminuria and endothelial dysfunction in essential hypertension. *Lancet* 1994;344:14-18.
- 62 Meinardi MT, Gietema JA, van der Graaf WTA, van Veldhuisen DJ, Runne MA, Sluiter WJ et al. Cardiovascular morbidity in long-term survivors of metastatic testicular cancer. *J Clin Oncol* 2000;18:1725-1732.
- 63 Bellmunt J, Navarro M, Morales S, Jolis L, Carulla J, Knobel H et al. Capillary microscopy is a potentially useful method for detecting bleomycin vascular toxicity. *Cancer* 1990;65:303-309.
- 64 Rayssiguier Y, Mbega JD, Durlach V, Gueux E, Durlach J, Giry J et al. Magnesium and blood pressure. I. Animal studies. *Magnes Res* 1992;5:139-146.
- 65 Durlach J, Durlach V, Rayssiguier Y, Bara M, Guet-Bara A. Magnesium and blood pressure. II. Clinical studies. *Magnes Res* 1992;5:147-153.
- 66 Vogelzang NJ, Torkelson JL, Kennedy BJ. Hypomagnesemia, renal dysfunction, and Raynaud's phenomenon in patients treated with cisplatin, vinblastine, and bleomycin. *Cancer* 1985;56:2765-2770.
- 67 Edwards GS, Lane M, Smith FE. Long-term treatment with cis-dichlorodiammineplatinum(II)-vinblastine-bleomycin: possible association with severe coronary artery disease [letter]. *Cancer Treat Rep* 1979;63:551-552.
- 68 Roth BJ, Greist A, Kubilis PS, Williams SD, Einhorn LH. Cisplatin-based combination chemotherapy for disseminated germ cell tumors: long-term follow-up. *J Clin Oncol* 1988;6:1239-1247.
- 69 Stoter G, Koopman A, Vendrik CP, Struyvenberg A, Sleijfer DTh, Williams B et al. Ten-year survival and late sequelae in testicular cancer patients treated with cisplatin, vinblastine, and bleomycin. *J Clin Oncol* 1989;7:1099-1104.
- 70 Belch J. Raynaud's phenomenon. *Cardiovasc Res* 1997;33:25-30.
- 71 Teutsch C, Lipton A, Harvey HA. Raynaud's phenomenon as a side effect of chemotherapy with vinblastine and bleomycin for testicular carcinoma. *Cancer Treat Rep* 1977;61:925-926.
- 72 Aass N, Kaasa S, Lund E, Kaalhus O, Heier MS, Fossa SD. Long-term somatic side-effects and morbidity in testicular cancer patients. *Br J Cancer* 1990;61:151-155.
- 73 Boyer M, Raghavan D, Harris PJ, Lietch J, Bleasel A, Walsh JC et al. Lack of late toxicity in patients treated with cisplatin-containing combination chemotherapy for metastatic testicular cancer. *J Clin Oncol* 1990;8:21-26.
- 74 Bissett D, Kunkeler L, Zwanenburg L, Paul J, Gray C, Swan IR et al. Long-term sequelae of treatment for testicular germ cell tumours. *Br J Cancer* 1990;62:655-659.
- 75 Berger CC, Bokemeyer C, Schneider M, Kuczyk MA, Schmoll HJ. Secondary Raynaud's phenomenon and other late vascular complications following chemotherapy for testicular cancer. *Eur J Cancer* 1995;31A:2229-2238.
- 76 Adoue D, Arlet P. Bleomycin and Raynaud's phenomenon [letter]. *Ann Intern Med* 1984;100:770.
- 77 Chernicoff DP, Bukowski RM, Young JR. Raynaud's phenomenon after bleomycin treatment [letter]. *Cancer Treat Rep* 1978;62:570-571.
- 78 Fossa SD, Lehne G, Heimdal K, Theodorsen L. Clinical and biochemical long-term toxicity after postoperative cisplatin-based chemotherapy in patients with low-stage testicular cancer. *Oncology* 1995;52:300-305.
- 79 Hansen SW, Olsen N. Raynaud's phenomenon in patients treated with cisplatin, vinblastine, and bleomycin for germ cell cancer: measurement of vasoconstrictor response to cold. *J Clin Oncol* 1989;7:940-942.

- 80 van Basten JP, Hoekstra HJ, van Driel MF, Schraffordt Koops H, Droste JH, Jonker-Pool G et al. Sexual dysfunction in nonseminoma testicular cancer patients is related to chemotherapy-induced angiopathy. *J Clin Oncol* 1997;15:2442-2448.
- 81 Hansen SW, Groth S, Daugaard G, Rossing N, Rorth M. Long-term effects on renal function and blood pressure of treatment with cisplatin, vinblastine, and bleomycin in patients with germ cell cancer. *J Clin Oncol* 1988;6:1728-1731.
- 82 Harrell RM, Sibley R, Vogelzang NJ. Renal vascular lesions after chemotherapy with vinblastine, bleomycin, and cisplatin. *Am J Med* 1982;73:429-433.
- 83 Wright LF, Reese EP, Bicknell SL. Renovascular hypertension after combination chemotherapy for testicular cancer. *J Urol* 1988;139:134-135.
- 84 Bosl GJ, Leitner SP, Atlas SA, Sealey JE, Preibisz JJ, Scheiner E. Increased plasma renin and aldosterone in patients treated with cisplatin-based chemotherapy for metastatic germ-cell tumors. *J Clin Oncol* 1986;4:1684-1689.
- 85 Wolf PA, D'Agostino RB, Belanger AJ, Kannel WB. Probability of stroke: a risk profile from the Framingham Study. *Stroke* 1991;22:312-318.
- 86 Umeki S. Decreases in serum cholesterol levels in advanced lung cancer. *Respiration* 1993;60:178-181.
- 87 Forones NM, Falcao JB, Mattos D, Barone B. Cholesterolemia in colorectal cancer. *Hepatogastroenterology* 1998;45:1531-1534.
- 88 Henriksson P, Eriksson M, Ericsson S, Rudling M, Stege R, Berglund L et al. Hypocholesterolaemia and increased elimination of low-density lipoproteins in metastatic cancer of the prostate. *Lancet* 1989;2:1178-1180.
- 89 Shiroeda O, Yamaguchi N, Kawai K. Stimulation of low density lipoprotein receptor activity by conditioned medium from a human cancer cell line. *Cancer Res* 1987;47:4630-4633.
- 90 Ueyama Y, Matsuzawa Y, Yamashita S, Funahashi T, Sakai N, Nakamura T et al. Hypocholesterolaemic factor from gallbladder cancer cells. *Lancet* 1990;336:707-709.
- 91 Berger CC, Bokemeyer C, Schuppert F, Schmoll HJ. Endocrinological late effects after chemotherapy for testicular cancer. *Br J Cancer* 1996;73:1108-1114.
- 92 Bosl GJ, Bajorunas D. Pituitary and testicular hormonal function after treatment for germ cell tumours. *Int J Androl* 1987;10:381-384.
- 93 Haffner SM, Mykkanen L, Valdez RA, Katz MS. Relationship of sex hormones to lipids and lipoproteins in nondiabetic men. *J Clin Endocrinol Metab* 1993;77:1610-1615.
- 94 Hansen SW, Berthelsen JG, von der Maase H. Long-term fertility and Leydig cell function in patients treated for germ cell cancer with cisplatin, vinblastine, and bleomycin versus surveillance. *J Clin Oncol* 1990;8:1695-1698.
- 95 Oppenheim DS, Greenspan SL, Zervas NT, Schoenfeld DA, Klibanski A. Elevated serum lipids in hypogonadal men with and without hyperprolactinemia. *Ann Intern Med* 1989;111:288-292.
- 96 Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW, Jr. Body-mass index and mortality in a prospective cohort of U.S. adults. *N Engl J Med* 1999;341:1097-1105.
- 97 Simon D, Preziosi P, Barrett-Connor E, Roger M, Saint-Paul M, Nahoul K et al. Interrelation between plasma testosterone and plasma insulin in healthy adult men: the Telecom Study. *Diabetologia* 1992;35:173-177.
- 98 Yudkin JS, Forrest RD, Jackson CA. Microalbuminuria as predictor of vascular disease in non-diabetic subjects. Islington Diabetes Survey. *Lancet* 1988;2:530-533.
- 99 Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988;37:1595-1607.
- 100 DeVita VT, Jr., Hubbard SM. Hodgkin's disease. *N Engl J Med* 1993;328:560-565.
- 101 Henry-Amar M, Joly F. Late complications after Hodgkin's disease. *Ann Oncol* 1996;7 Suppl 4:115-126.
- 102 DeVita VT, Jr., Serpick AA, Carbone PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. *Ann Intern Med* 1970;73:881-895.
- 103 Bonadonna G, Santoro A. ABVD chemotherapy in the treatment of Hodgkin's disease. *Cancer Treat Rev* 1982;9:21-35.
- 104 Fisher RI, Gaynor ER, Dahlberg S, Oken MM, Grogan TM, Mize EM et al. Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. *N Engl J Med* 1993;328:1002-1006.
- 105 Miller TP, Dahlberg S, Cassady JR, Adelstein DJ, Spier CM, Grogan TM et al. Chemotherapy alone compared with chemotherapy plus radiotherapy for localized intermediate- and high-grade non-Hodgkin's lymphoma. *N Engl J Med* 1998;339:21-26.

- 106 Klencke B, Kaplan L. Advances and future challenges in non-Hodgkin's lymphoma. *Curr Opin Oncol* 1998;10:422-427.
- 107 Bristow MR, Billingham ME, Mason JW, Daniels JR. Clinical spectrum of anthracycline antibiotic cardiotoxicity. *Cancer Treat Rep* 1978;62:873-879.
- 108 Laufman LR, Jones JJ, Morrice B, Han CH, Halk D, Fisher B. Case report of a lethal cardiac toxic effect following high-dose cyclophosphamide [letter]. *J Natl Cancer Inst* 1995;87:539-540.
- 109 Fraiser LH, Kanekal S, Kehrler JP. Cyclophosphamide toxicity. Characterising and avoiding the problem. *Drugs* 1991;42:781-795.
- 110 Tulleken JE, Kooiman CG, van der Werf TS, Zijlstra JG, de Vries EGE. Constrictive pericarditis after high-dose chemotherapy [letter]. *Lancet* 1997;350:1601.
- 111 Gottdiener JS, Appelbaum FR, Ferrans VJ, Deisseroth A, Ziegler J. Cardiotoxicity associated with high-dose cyclophosphamide therapy. *Arch Intern Med* 1981;141:758-763.
- 112 Steinherz LJ, Steinherz PG, Mangiacasale D, O'Reilly R, Allen J, Sorell M et al. Cardiac changes with cyclophosphamide. *Med Pediatr Oncol* 1981;9:417-422.
- 113 Isberg B, Paul C, Jonsson L, Svahn U. Myocardial toxicity of high-dose cyclophosphamide in rabbits treated with daunorubicin. *Cancer Chemother Pharmacol* 1991;28:171-180.
- 114 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.
- 115 Cazin B, Gorin NC, Laporte JP, Gallet B, Douay L, Lopez M et al. Cardiac complications after bone marrow transplantation. A report on a series of 63 consecutive transplantations. *Cancer* 1986;57:2061-2069.
- 116 House KW, Simon SR, Pugh RP. Chemotherapy-induced myocardial infarction in a young man with Hodgkin's disease. *Clin Cardiol* 1992;15:122-125.
- 117 Scholz KH, Herrmann C, Tebbe U, Chemnitz JM, Helmchen U, Kreuzer H. Myocardial infarction in young patients with Hodgkin's disease--potential pathogenic role of radiotherapy, chemotherapy, and splenectomy. *Clin Invest* 1993;71:57-64.
- 118 Hirsch A, Vander EN, Straus DJ, Gomez EG, Leung D, Portlock CS et al. Effect of ABVD chemotherapy with and without mantle or mediastinal irradiation on pulmonary function and symptoms in early-stage Hodgkin's disease. *J Clin Oncol* 1996;14:1297-1305.
- 119 Seifter EJ, Young RC, Longo DL. Deep venous thrombosis during therapy for Hodgkin's disease. *Cancer Treat Rep* 1985;69:1011-1013.
- 120 Clarke CS, Otridge BW, Carney DN. Thromboembolism. A complication of weekly chemotherapy in the treatment of non-Hodgkin's lymphoma. *Cancer* 1990;66:2027-2030.
- 121 Gottlieb SL, Edmiston WA, Jr., Haywood LJ. Late, late doxorubicin cardiotoxicity. *Chest* 1980;78:880-882.
- 122 Grenier MA, Lipshultz SE. Epidemiology of anthracycline cardiotoxicity in children and adults. *Semin Oncol* 1998;25:72-85.
- 123 Ghielmini M, Zappa F, Menafoglio A, Caoduro L, Pampallona S, Gallino A. The high-dose sequential (Milan) chemotherapy/PBSC transplantation regimen for patients with lymphoma is not cardiotoxic. *Ann Oncol* 1999;10:533-537.
- 124 Santoro A, Bonadonna G, Valagussa P, Zucali R, Viviani S, Villani F et al. Long-term results of combined chemotherapy-radiotherapy approach in Hodgkin's disease: superiority of ABVD plus radiotherapy versus MOPP plus radiotherapy. *J Clin Oncol* 1987;5:27-37.
- 125 Viviani S, Bonadonna G, Santoro A, Bonfante V, Zanini M, Devizzi L et al. Alternating versus hybrid MOPP and ABVD combinations in advanced Hodgkin's disease: ten-year results. *J Clin Oncol* 1996;14:1421-1430.
- 126 Salloum E, Tanoue LT, Wackers FJ, Zelterman D, Hu GL, Cooper DL. Assessment of cardiac and pulmonary function in adult patients with Hodgkin's disease treated with ABVD or MOPP/ABVD plus adjuvant low-dose mediastinal irradiation. *Cancer Invest* 1999;17:171-180.
- 127 Aviles A, Arevila N, Diaz Maqueo JC, Gomez T, Garcia R, Nambo MJ. Late cardiac toxicity of doxorubicin, epirubicin, and mitoxantrone therapy for Hodgkin's disease in adults. *Leuk Lymphoma* 1993;11:275-279.
- 128 Haddy TB, Adde MA, McCalla J, Domanski MJ, Datiles M, III, Meehan SC et al. Late effects in long-term survivors of high-grade non-Hodgkin's lymphomas. *J Clin Oncol* 1998;16:2070-2079.
- 129 Freter CE, Lee TC, Billingham ME, Chak L, Bristow MR. Doxorubicin cardiac toxicity manifesting seven years after treatment. Case report and review. *Am J Med* 1986;80:483-485.
- 130 Minow RA, Benjamin RS, Lee ET, Gottlieb JA. Adriamycin cardiomyopathy--risk factors. *Cancer* 1977;39:1397-1402.

- 131 LaMonte CS, Yeh SD, Straus DJ. Long-term follow-up of cardiac function in patients with Hodgkin's disease treated with mediastinal irradiation and combination chemotherapy including doxorubicin. *Cancer Treat Rep* 1986;70:439-444.
- 132 Brice P, Tredaniel J, Monsuez JJ, Marolleau JP, Ferme C, Hennequin C et al. Cardiopulmonary toxicity after three courses of ABVD and mediastinal irradiation in favorable Hodgkin's disease. *Ann Oncol* 1991;2 Suppl 2:73-76.
- 133 Benoff LJ, Schweitzer P. Radiation therapy-induced cardiac injury. *Am Heart J* 1995;129:1193-1196.
- 134 Hancock SL, Tucker MA, Hoppe RT. Factors affecting late mortality from heart disease after treatment of Hodgkin's disease. *JAMA* 1993;270:1949-1955.
- 135 Boivin JF, Hutchison GB, Lubin JH, Mauch P. Coronary artery disease mortality in patients treated for Hodgkin's disease. *Cancer* 1992;69:1241-1247.
- 136 Henry-Amar M, Hayat M, Meerwaldt JH, Burgers M, Carde P, Somers R et al. Causes of death after therapy for early stage Hodgkin's disease entered on EORTC protocols. EORTC Lymphoma Cooperative Group. *Int J Radiat Oncol Biol Phys* 1990;19:1155-1157.
- 137 Wagenvoort CA. Pulmonary veno-occlusive disease. Entity or syndrome? *Chest* 1976;69:82-86.
- 138 Lombard CM, Churg A, Winokur S. Pulmonary veno-occlusive disease following therapy for malignant neoplasms. *Chest* 1987;92:871-876.
- 139 Rose AG. Pulmonary veno-occlusive disease due to bleomycin therapy for lymphoma. Case reports. *S Afr Med J* 1983;64:636-638.
- 140 Gordon T, Kannel WB, Hjortland MC, McNamara PM. Menopause and coronary heart disease. The Framingham Study. *Ann Intern Med* 1978;89:157-161.
- 141 Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999;340:1801-1811.
- 142 Sourander L, Rajala T, Raiha I, Makinen J, Erkkola R, Helenius H. Cardiovascular and cancer morbidity and mortality and sudden cardiac death in postmenopausal women on oestrogen replacement therapy (ERT). *Lancet* 1998;352:1965-1969.
- 143 Suda Y, Ohta H, Makita K, Takamatsu K, Horiguchi F, Nozawa S. Influence of bilateral oophorectomy upon lipid metabolism. *Maturitas* 1998;29:147-154.
- 144 Farish E, Fletcher CD, Hart DM, Smith ML. Effects of bilateral oophorectomy on lipoprotein metabolism. *Br J Obstet Gynaecol* 1990;97:78-82.
- 145 Bruschi F, Meschia M, Soma M, Perotti D, Paoletti R, Crosignani PG. Lipoprotein(a) and other lipids after oophorectomy and estrogen replacement therapy. *Obstet Gynecol* 1996;88:950-954.
- 146 Munker R, Cremer P, Fries S, Hiller E. Serum lipids in patients with Hodgkin's disease in complete remission. *Acta Oncol* 1997;36:75-77.
- 147 Bonadonna G, Brusamolino E, Valagussa P, Rossi A, Brugnatelli L, Brambilla C et al. Combination chemotherapy as an adjuvant treatment in operable breast cancer. *N Engl J Med* 1976;294:405-410.
- 148 Buzdar AU, Kau SW, Smith TL, Hortobagyi GN. Ten-year results of FAC adjuvant chemotherapy trial in breast cancer. *Am J Clin Oncol* 1989;12:123-128.
- 149 Fisher B, Brown AM, Dimitrov NV, Poisson R, Redmond C, Margolese RG et al. Two months of doxorubicin-cyclophosphamide with and without interval reinduction therapy compared with 6 months of cyclophosphamide, methotrexate, and fluorouracil in positive-node breast cancer patients with tamoxifen-nonresponsive tumors: results from the National Surgical Adjuvant Breast and Bowel Project B-15. *J Clin Oncol* 1990;8:1483-1496.
- 150 Coombes RC, Bliss JM, Wils J, Morvan F, Espie M, Amadori D et al. Adjuvant cyclophosphamide, methotrexate, and fluorouracil versus fluorouracil, epirubicin, and cyclophosphamide chemotherapy in premenopausal women with axillary node-positive operable breast cancer: results of a randomized trial. The International Collaborative Cancer Group. *J Clin Oncol* 1996;14:35-45.
- 151 Sasson Z, Morgan CD, Wang B, Thomas G, MacKenzie B, Platts ME. 5-Fluorouracil related toxic myocarditis: case reports and pathological confirmation. *Can J Cardiol* 1994;10:861-864.
- 152 Millart H, Brabant L, Lorenzato M, Lamiable D, Albert O, Choisy H. The effects of 5-fluorouracil on contractility and oxygen uptake of the isolated perfused rat heart. *Anticancer Res* 1992;12:571-576.
- 153 Chaudary S, Song SY, Jaski BE. Profound, yet reversible, heart failure secondary to 5-fluorouracil. *Am J Med* 1988;85:454-456.
- 154 Jakubowski AA, Kemeny N. Hypotension as a manifestation of cardiotoxicity in three patients receiving cisplatin and 5-fluorouracil. *Cancer* 1988;62:266-269.

- 155 Schober C, Papageorgiou E, Harstrick A, Bokemeyer C, Mugge A, Stahl M et al. Cardiotoxicity of 5-fluorouracil in combination with folinic acid in patients with gastrointestinal cancer. *Cancer* 1993;72:2242-2247.
- 156 Coronel B, Madonna O, Mercatello A, Caillette A, Moskovtchenko JF. Myocardiototoxicity of 5 fluorouracil. *Intensive Care Med* 1988;14:429-430.
- 157 Weidmann B, Jansen W, Heider A, Niederle N. 5-Fluorouracil cardiotoxicity with left ventricular dysfunction under different dosing regimens. *Am J Cardiol* 1995;75:194-195.
- 158 Gradishar WJ, Vokes EE. 5-Fluorouracil cardiotoxicity: a critical review. *Ann Oncol* 1990;1:409-414.
- 159 de Forni M, Malet-Martino MC, Jaillais P, Shubinski RE, Bachaud JM, Lemaire L et al. Cardiotoxicity of high-dose continuous infusion fluorouracil: a prospective clinical study. *J Clin Oncol* 1992;10:1795-1801.
- 160 Cwikiel M, Eskilsson J, Albertsson M, Stavenow L. The influence of 5-fluorouracil and methotrexate on vascular endothelium. An experimental study using endothelial cells in the culture. *Ann Oncol* 1996;7:731-737.
- 161 Robben NC, Pippas AW, Moore JO. The syndrome of 5-fluorouracil cardiotoxicity. An elusive cardiopathy. *Cancer* 1993;71:493-509.
- 162 Lomeo AM, Avolio C, Iacobellis G, Manzione L. 5-Fluorouracil cardiotoxicity. *Eur J Gynaecol Oncol* 1990;11:237-241.
- 163 Levine MN, Gent M, Hirsh J, Arnold A, Goodyear MD, Hryniuk W et al. The thrombogenic effect of anticancer drug therapy in women with stage II breast cancer. *N Engl J Med* 1988;318:404-407.
- 164 Weiss RB, Tormey DC, Holland JF, Weinberg VE. Venous thrombosis during multimodal treatment of primary breast carcinoma. *Cancer Treat Rep* 1981;65:677-679.
- 165 Nicolson GL, Custead SE. Effects of chemotherapeutic drugs on platelet and metastatic tumor cell-endothelial cell interactions as a model for assessing vascular endothelial integrity. *Cancer Res* 1985;45:331-336.
- 166 Buzdar AU, Marcus C, Smith TL, Blumenschein GR. Early and delayed clinical cardiotoxicity of doxorubicin. *Cancer* 1985;55:2761-2765.
- 167 Shapiro CL, Hardenbergh PH, Gelman R, Blanks D, Hauptman P, Recht A et al. Cardiac effects of adjuvant doxorubicin and radiation therapy in breast cancer patients. *J Clin Oncol* 1998;16:3493-3501.
- 168 Gianni L, Zambetti M, Moliterni A, Stefanelli M, Materazzo C, Cipriani S et al. Cardiac Sequelae in Operable Breast Cancer Patients After CMF ± Doxorubicin (A) ± Irradiation. *Proc Am Soc Clin Oncol* 1999; abstract 255.
- 169 Cuzick J, Stewart H, Peto R, Baum M, Fisher B, Host H et al. Overview of randomized trials of postoperative adjuvant radiotherapy in breast cancer. *Cancer Treat Rep* 1987;71:15-29.
- 170 Cuzick J, Stewart H, Rutqvist L, Houghton J, Edwards R, Redmond C et al. Cause-specific mortality in long-term survivors of breast cancer who participated in trials of radiotherapy. *J Clin Oncol* 1994;12:447-453.
- 171 Paszat LF, Mackillop WJ, Groome PA, Boyd C, Schulze K, Holowaty E. Mortality from myocardial infarction after adjuvant radiotherapy for breast cancer in the surveillance, epidemiology, and end-results cancer registries. *J Clin Oncol* 1998;16:2625-2631.
- 172 Fuller SA, Haybittle JL, Smith RE, Dobbs HJ. Cardiac doses in post-operative breast irradiation. *Radiother Oncol* 1992;25:19-24.
- 173 Rutqvist LE, Liedberg A, Hammar N, Dalberg K. Myocardial infarction among women with early-stage breast cancer treated with conservative surgery and breast irradiation. *Int J Radiat Oncol Biol Phys* 1998;40:359-363.
- 174 Højris I, Overgaard M, Christensen JJ, Overgaard J. Morbidity and mortality of ischaemic heart disease in high-risk breast-cancer patients after adjuvant postmastectomy systemic treatment with or without radiotherapy: analysis of DBCG 82b and 82c randomised trials. Radiotherapy Committee of the Danish Breast Cancer Cooperative Group. *Lancet* 1999;354:1425-1430.
- 175 Valagussa P, Zambetti M, Biasi S, Moliterni A, Zucali R, Bonadonna G. Cardiac effects following adjuvant chemotherapy and breast irradiation in operable breast cancer. *Ann Oncol* 1994;5:209-216.
- 176 Blomqvist C, Tiusanen K, Elomaa I, Rissanen P, Hietanen T, Heinonen E et al. The combination of radiotherapy, adjuvant chemotherapy (cyclophosphamide-doxorubicin-5-fluorouracil) and tamoxifen in stage II breast cancer. Long-term follow-up results of a randomised trial. *Br J Cancer* 1992;66:1171-1176.
- 177 Bines J, Oleske DM, Cobleigh MA. Ovarian function in premenopausal women treated with adjuvant chemotherapy for breast cancer. *J Clin Oncol* 1996;14:1718-1729.
- 178 Saarto T, Blomqvist C, Ehnholm C, Taskinen MR, Elomaa I. Effects of chemotherapy-induced castration on serum lipids and apoproteins in premenopausal women with node-positive breast cancer. *J Clin Endocrinol Metab* 1996;81:4453-4457.

- 179 Love RR, Newcomb PA, Wiebe DA, Surawicz TS, Jordan VC, Carbone PP et al. Effects of tamoxifen therapy on lipid and lipoprotein levels in postmenopausal patients with node-negative breast cancer. *J Natl Cancer Inst* 1990;82:1327-1332.
- 180 Love RR, Wiebe DA, Feyzi JM, Newcomb PA, Chappell RJ. Effects of tamoxifen on cardiovascular risk factors in postmenopausal women after 5 years of treatment. *J Natl Cancer Inst* 1994;86:1534-1539.
- 181 McDonald CC, Alexander FE, Whyte BW, Forrest AP, Stewart HJ. Cardiac and vascular morbidity in women receiving adjuvant tamoxifen for breast cancer in a randomised trial. The Scottish Cancer Trials Breast Group. *BMJ* 1995;311:977-980.
- 182 Rutqvist LE, Mattsson A. Cardiac and thromboembolic morbidity among postmenopausal women with early-stage breast cancer in a randomized trial of adjuvant tamoxifen. The Stockholm Breast Cancer Study Group. *J Natl Cancer Inst* 1993;85:1398-1406.
- 183 Hozumi Y, Kawano M, Saito T, Miyata M. Effect of tamoxifen on serum lipid metabolism. *J Clin Endocrinol Metab* 1998;83:1633-1635.
- 184 Thangaraju M, Kumar K, Gandhirajan R, Sachdanandam P. Effect of tamoxifen on plasma lipids and lipoproteins in postmenopausal women with breast cancer. *Cancer* 1994;73:659-663.
- 185 Colls BM, George PM. Severe hypertriglyceridaemia and hypercholesterolaemia associated with tamoxifen use. *Clin Oncol (R Coll Radiol)* 1998;10:270-271.
- 186 Hozumi Y, Kawano M, Miyata M. Severe hypertriglyceridemia caused by tamoxifen-treatment after breast cancer surgery. *Endocr* 1997;44:745-749.
- 187 Jonkers GH. Tamoxifen and hypertriglyceridemia [letter]. *Neth J Med* 1999;55:84-85.
- 188 Prolongation of the disease-free interval in surgically treated rectal carcinoma. Gastrointestinal Tumor Study Group. *N Engl J Med* 1985;312:1465-1472.
- 189 Fisher B, Wolmark N, Rockette H, Redmond C, Deutsch M, Wickerham DL et al. Postoperative adjuvant chemotherapy or radiation therapy for rectal cancer: results from NSABP protocol R-01. *J Natl Cancer Inst* 1988;80:21-29.
- 190 Grandi AM, Pinotti G, Morandi E, Zanzi P, Bulgheroni P, Guasti L et al. Noninvasive evaluation of cardiotoxicity of 5-fluorouracil and low doses of folinic acid: a one-year follow-up study. *Ann Oncol* 1997;8:705-708.
- 191 Papamichael D, Amft N, Slevin ML, D'Cruz D. 5-Fluorouracil-induced Raynaud's phenomenon [letter]. *Eur J Cancer* 1998;34:1983.
- 192 Gianni L, Munzone E, Capri G,ulfaro F, Tarenzi E, Villani F et al. Paclitaxel by 3-hour infusion in combination with bolus doxorubicin in women with untreated metastatic breast cancer: high antitumor efficacy and cardiac effects in a dose-finding and sequence-finding study. *J Clin Oncol* 1995;13:2688-2699.
- 193 Gehl J, Boesgaard M, Paaske T, Vittrup JB, Dombernowsky P. Combined doxorubicin and paclitaxel in advanced breast cancer: effective and cardiotoxic. *Ann Oncol* 1996;7:687-693.
- 194 Ewer MS, Gibbs HR, Swafford J, Benjamin RS. Cardiotoxicity in patients receiving trastuzumab (Herceptin): primary toxicity, synergistic or sequential stress, or surveillance artifact? *Semin Oncol* 1999;26:96-101.
- 195 de Wit R, Stoter G, Kaye SB, Sleijfer DTh, Jones WG, ten Bokkel H et al. Importance of bleomycin in combination chemotherapy for good-prognosis testicular nonseminoma: a randomized study of the European Organization for Research and Treatment of Cancer Genitourinary Tract Cancer Cooperative Group. *J Clin Oncol* 1997;15:1837-1843.
- 196 Alberts DS, Garcia DJ. Safety aspects of pegylated liposomal doxorubicin in patients with cancer. *Drugs* 1997;54 Suppl 4:30-35.
- 197 Horwich A, Sleijfer DTh, Fossa SD, Kaye SB, Oliver RT, Cullen MH et al. Randomized trial of bleomycin, etoposide, and cisplatin compared with bleomycin, etoposide, and carboplatin in good-prognosis metastatic non-seminomatous germ cell cancer: a Multiinstitutional Medical Research Council/European Organization for Research and Treatment of Cancer Trial. *J Clin Oncol* 1997;15:1844-1852.
- 198 Hensley ML, Schuchter LM, Lindley C, Meropol NJ, Cohen GI, Broder G et al. American Society of Clinical Oncology Clinical Practice Guidelines for the Use of Chemotherapy and Radiotherapy Protectants. *J Clin Oncol* 1999;17:3333-3355.
- 199 Schwartz RG, McKenzie WB, Alexander J, Sager P, D'Souza A, Manatunga A et al. Congestive heart failure and left ventricular dysfunction complicating doxorubicin therapy. Seven-year experience using serial radionuclide angiocardiology. *Am J Med* 1987;82:1109-1118.
- 200 Steinherz LJ, Graham T, Hurwitz R, Sondheimer HM, Schwartz RG, Shaffer EM 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.

- 201 Meinardi MT, van der Graaf WTA, van Veldhuisen DJ, Gietema JA, de Vries EGE, Sleijfer DTh. Detection of anthracycline-induced cardiotoxicity. *Cancer Treat Rev* 1999;25:237-247.
- 202 Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The SOLVD Investigators. *N Engl J Med* 1992;327:685-691.
- 203 Exner DV, Dries DL, Waclawiw MA, Shelton B, Domanski MJ. Beta-adrenergic blocking agent use and mortality in patients with asymptomatic and symptomatic left ventricular systolic dysfunction: a post hoc analysis of the Studies of Left Ventricular Dysfunction. *J Am Coll Cardiol* 1999;33:916-923.
- 204 Walsh BW, Kuller LH, Wild RA, Paul S, Farmer M, Lawrence JB et al. Effects of raloxifene on serum lipids and coagulation factors in healthy postmenopausal women. *JAMA* 1998;279:1445-1451.
- 205 Bristow MR, Thompson PD, Martin RP, Mason JW, Billingham ME, Harrison DC. Early anthracycline cardiotoxicity. *Am J Med* 1978;65:823-832.
- 206 Bleyer WA. The impact of childhood cancer on the United States and the world. *CA Cancer J Clin* 1990;40:355-367.
- 207 Steinberg JS, Cohen AJ, Wasserman AG, Cohen P, Ross AM. Acute arrhythmogenicity of doxorubicin administration. *Cancer* 1987;60:1213-1218.
- 208 Praga C, Beretta G, Vigo PL, Lenaz GR, Pollini C, Bonadonna G et al. Adriamycin cardiotoxicity: a survey of 1273 patients. *Cancer Treat Rep* 1979;63:827-834.
- 209 Ferrans VJ. Overview of cardiac pathology in relation to anthracycline cardiotoxicity. *Cancer Treat Rep* 1978;62:955-961.
- 210 Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity. *Cancer* 1973;32:302-314.
- 211 Jain KK, Casper ES, Geller NL, Hakes TB, Kaufman RJ, Currie V et al. A prospective randomized comparison of epirubicin and doxorubicin in patients with advanced breast cancer. *J Clin Oncol* 1985;3:818-826.
- 212 Nielsen D, Jensen JB, Dombernowsky P, Munck O, Fogh J, Brynjolf I et al. Epirubicin cardiotoxicity: a study of 135 patients with advanced breast cancer. *J Clin Oncol* 1990;8:1806-1810.
- 213 Ryberg M, Nielsen D, Skovsgaard T, Hansen J, Jensen BV, Dombernowsky P. Epirubicin cardiotoxicity: an analysis of 469 patients with metastatic breast cancer. *J Clin Oncol* 1998;16:3502-3508.
- 214 Henderson IC, Allegra JC, Woodcock T, Wolff S, Bryan S, Cartwright K et al. Randomized clinical trial comparing mitoxantrone with doxorubicin in previously treated patients with metastatic breast cancer. *J Clin Oncol* 1989;7:560-571.
- 215 Torti FM, Bristow MR, Howes AE, Aston D, Stockdale FE, Carter SK et al. Reduced cardiotoxicity of doxorubicin delivered on a weekly schedule. Assessment by endomyocardial biopsy. *Ann Intern Med* 1983;99:745-749.
- 216 Legha SS, Benjamin RS, Mackay B, Ewer M, Wallace S, Valdivieso M et al. Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. *Ann Intern Med* 1982;96:133-139.
- 217 Pratt CB, Ransom JL, Evans WE. Age-related adriamycin cardiotoxicity in children. *Cancer Treat Rep* 1978;62:1381-1385.
- 218 Schaadt B, Kelbaek H. Age and left ventricular ejection fraction identify patients with advanced breast cancer at high risk for development of epirubicin-induced heart failure. *J Nucl Cardiol* 1997;4:494-501.
- 219 Silber JH, Jakacki RI, Larsen RL, Goldwein JW, Barber G. Increased risk of cardiac dysfunction after anthracyclines in girls. *Med Pediatr Oncol* 1993;21:477-479.
- 220 Villani F, Comazzi R, Lacaita G, Guindani A, Genitoni V, Volonterio A et al. Possible enhancement of the cardiotoxicity of doxorubicin when combined with mitomycin C. *Med Oncol Tumor Pharmacother* 1985;2:93-97.
- 221 Doroshow JH. Effect of anthracycline antibiotics on oxygen radical formation in rat heart. *Cancer Res* 1983;43:460-472.
- 222 Doroshow JH. Anthracycline antibiotic-stimulated superoxide, hydrogen peroxide, and hydroxyl radical production by NADH dehydrogenase. *Cancer Res* 1983;43:4543-4551.
- 223 Rajagopalan S, Politi PM, Sinha BK, Myers CE. Adriamycin-induced free radical formation in the perfused rat heart: implications for cardiotoxicity. *Cancer Res* 1988;48:4766-4769.
- 224 Jackson JA, Reeves JP, Muntz KH, Kruk D, Prough RA, Willerson JT et al. Evaluation of free radical effects and catecholamine alterations in adriamycin cardiotoxicity. *Am J Pathol* 1984;117:140-153.
- 225 Singal PK, Pierce GN. Adriamycin stimulates low-affinity Ca²⁺ binding and lipid peroxidation but depresses myocardial function. *Am J Physiol* 1986;250:H419-425.
- 226 Kusuoka H, Futaki S, Koretsune Y, Kitabatake A, Suga H, Kamada T et al. Alterations of intracellular calcium homeostasis and myocardial energetics in acute adriamycin-induced heart failure. *J Cardiovasc Pharmacol* 1991;18:437-444.

- 227 Holmberg SR, Williams AJ. Patterns of interaction between anthraquinone drugs and the calcium- release channel from cardiac sarcoplasmic reticulum. *Circ Res* 1990;67:272-283.
- 228 Takano H, Ozawa H, Kobayashi I, Hamaoka S, Nakajima J, Nakamura T et al. Myocardial sympathetic dysinnervation in doxorubicin cardiomyopathy. *J Cardiol* 1996;27:49-55.
- 229 Lekakis J, Prassopoulos V, Athanassiadis P, Kostamis P, Mouloupoulos S. Doxorubicin-induced cardiac neurotoxicity: study with iodine 123- labeled metaiodobenzylguanidine scintigraphy. *J Nucl Cardiol* 1996;3:37-41.
- 230 Fujita N, Hiroe M, Ohta Y, Horie T, Hosoda S. Chronic effects of metoprolol on myocardial beta-adrenergic receptors in doxorubicin-induced cardiac damage in rats. *J Cardiovasc Pharmacol* 1991;17:656-661.
- 231 Tong J, Ganguly PK, Singal PK. Myocardial adrenergic changes at two stages of heart failure due to adriamycin treatment in rats. *Am J Physiol* 1991;260:H909-916.
- 232 Eichhorn EJ, Bristow MR. Medical therapy can improve the biological properties of the chronically failing heart. A new era in the treatment of heart failure. *Circulation* 1996;94:2285-2296.
- 233 Wilkins MR, Redondo J, Brown LA. The natriuretic-peptide family. *Lancet* 1997;349:1307-1310.
- 234 Ganz WI, Sridhar KS, Forness TJ. Detection of early anthracycline cardiotoxicity by monitoring the peak filling rate. *Am J Clin Oncol* 1993;16:109-112.
- 235 Isner JM, Ferrans VJ, Cohen SR, Witkind BG, Virmani R, Gottdiener JS et al. Clinical and morphologic cardiac findings after anthracycline chemotherapy. Analysis of 64 patients studied at necropsy. *Am J Cardiol* 1983;51:1167-1174.
- 236 Sparano JA. Use of dexrazoxane and other strategies to prevent cardiomyopathy associated with doxorubicin-taxane combinations. *Semin Oncol* 1998;25:66-71.
- 237 Lemez P, Maresova J. Efficacy of dexrazoxane as a cardioprotective agent in patients receiving mitoxantrone- and daunorubicin-based chemotherapy. *Semin Oncol* 1998;25:61-65.
- 238 Singal PK, Siveski-Illkovic N, Hill M, Thomas TP, Li T. Combination therapy with probucol prevents adriamycin-induced cardiomyopathy. *J Mol Cell Cardiol* 1995;27:1055-1063.
- 239 Druck MN, Gulenchyn KY, Evans WK, Gotlieb A, Srigley JR, Bar-Shlomo BZ et al. Radionuclide angiography and endomyocardial biopsy in the assessment of doxorubicin cardiotoxicity. *Cancer* 1984;53:1667-1674.
- 240 Lenaz L, Page JA. Cardiotoxicity of adriamycin and related anthracyclines. *Cancer Treat Rev* 1976;3:111-120.
- 241 Ali MK, Soto A, Maroongroge D, Bekheit-Saad S, Buzdar AU, Blumenschein GR et al. Electrocardiographic changes after adriamycin chemotherapy. *Cancer* 1979;43:465-471.
- 242 Minow RA, Benjamin RS, Lee ET, Gottlieb JA. QRS voltage change with adriamycin administration. *Cancer Treat Rep* 1978;62:931-934.
- 243 Larsen RL, Jakacki RI, Vetter VL, Meadows AT, Silber JH, Barber G. Electrocardiographic changes and arrhythmias after cancer therapy in children and young adults. *Am J Cardiol* 1992;70:73-77.
- 244 Schwartz CL, Hobbie WL, Truesdell S, Constine LC, Clark EB. Corrected QT interval prolongation in anthracycline-treated survivors of childhood cancer. *J Clin Oncol* 1993;11:1906-1910.
- 245 Ferrari S, Figus E, Cagnano R, Iantorno D, Bacci G. The role of corrected QT interval in the cardiology follow-up of young patients treated with Adriamycin. *J Chemother* 1996;8:232-236.
- 246 Ganz WI, Sridhar KS, Ganz SS, Gonzalez R, Chakko S, Serafini A. Review of tests for monitoring doxorubicin-induced cardiomyopathy. *Oncology* 1996;53:461-470.
- 247 Alexander J, Dainiak N, Berger HJ, Goldman L, Johnstone D, Reduto L et al. Serial assessment of doxorubicin cardiotoxicity with quantitative radionuclide angiography. *N Engl J Med* 1979;300:278-283.
- 248 Gottdiener JS, Mathisen DJ, Borer JS, Bonow RO, Myers CE, Barr LH et al. Doxorubicin cardiotoxicity: assessment of late left ventricular dysfunction by radionuclide cineangiography. *Ann Intern Med* 1981;94:430-435.
- 249 Ritchie JL, Singer JW, Thorning D, Sorensen SG, Hamilton GW. Anthracycline cardiotoxicity: clinical and pathologic outcomes assessed by radionuclide ejection fraction. *Cancer* 1980;46:1109-1116.
- 250 Palmeri ST, Bonow RO, Myers CE, Seipp C, Jenkins J, Green MV et al. Prospective evaluation of doxorubicin cardiotoxicity by rest and exercise radionuclide angiography. *Am J Cardiol* 1986;58:607-613.
- 251 Hurwitz RA, Siddiqui A, Caldwell RL, Weetman RM, Girod DA. Assessment of ventricular function in infants and children. Response to dobutamine infusion. *Clin Nucl Med* 1990;15:556-559.
- 252 Kuecherer HF, Ruffmann K, Schaefer E, Kuebler W. Doppler echocardiographic assessment of left ventricular filling dynamics in patients with coronary heart disease and normal systolic function. *Eur Heart J* 1988;9:649-656.
- 253 Zarich SW, Arbuckle BE, Cohen LR, Roberts M, Nesto RW. Diastolic abnormalities in young asymptomatic diabetic patients assessed by pulsed Doppler echocardiography. *J Am Coll Cardiol* 1988;12:114-120.
- 254 Lee BH, Goodenday LS, Muswick GJ, Yasnoff WA, Leighton RF, Skeel RT. Alterations in left ventricular diastolic function with doxorubicin therapy. *J Am Coll Cardiol* 1987;9:184-188.

- 255 Marchandise B, Schroeder E, Bosly A, Doyen C, Weynants P, Kremer R et al. Early detection of doxorubicin cardiotoxicity: interest of Doppler echocardiographic analysis of left ventricular filling dynamics. *Am Heart J* 1989;118:92-98.
- 256 Nishimura RA, Tajik AJ. Evaluation of diastolic filling of left ventricle in health and disease: Doppler echocardiography is the clinician's Rosetta Stone. *J Am Coll Cardiol* 1997;30:8-18.
- 257 Hutter JJ, Jr., Sahn DJ, Woolfenden JM, Carnahan Y. Evaluation of the cardiac effects of doxorubicin by serial echocardiography. *Am J Dis Child* 1981;135:653-657.
- 258 Nysom K, Holm K, Lipsitz SR, Mone SM, Colan SD, Orav EJ et al. Relationship between cumulative anthracycline dose and late cardiotoxicity in childhood acute lymphoblastic leukemia. *J Clin Oncol* 1998;16:545-550.
- 259 Sorensen K, Levitt G, Bull C, Chessells J, Sullivan I. Anthracycline dose in childhood acute lymphoblastic leukemia: issues of early survival versus late cardiotoxicity. *J Clin Oncol* 1997;15:61-68.
- 260 Jakacki RI, Larsen RL, Barber G, Heyman S, Fridman M, Silber JH. Comparison of cardiac function tests after anthracycline therapy in childhood. Implications for screening. *Cancer* 1993;72:2739-2745.
- 261 Giannuzzi P, Temporelli PL, Bosimini E, Silva P, Imparato A, Corra U et al. Independent and incremental prognostic value of Doppler-derived mitral deceleration time of early filling in both symptomatic and asymptomatic patients with left ventricular dysfunction. *J Am Coll Cardiol* 1996;28:383-390.
- 262 Werner GS, Schaefer C, Dirks R, Figulla HR, Kreuzer H. Prognostic value of Doppler echocardiographic assessment of left ventricular filling in idiopathic dilated cardiomyopathy. *Am J Cardiol* 1994;73:792-798.
- 263 Cohen GI, Pietrolungo JF, Thomas JD, Klein AL. A practical guide to assessment of ventricular diastolic function using Doppler echocardiography. *J Am Coll Cardiol* 1996;27:1753-1760.
- 264 Tjeerdsma G, Meinardi MT, Der Graaf WTA, van den Berg MP, Mulder NH, Crijns HJGM et al. Early detection of anthracycline induced cardiotoxicity in asymptomatic patients with normal left ventricular systolic function: autonomic versus echocardiographic variables. *Heart* 1999;81:419-423.
- 265 Ewer MS, Ali MK, Gibbs HR, Swafford J, Graff KL, Cangir A et al. Cardiac diastolic function in pediatric patients receiving doxorubicin. *Acta Oncol* 1994;33:645-649.
- 266 Sung RY, Huang GY, Shing MK, Oppenheimer SJ, Li CK, Lau J et al. Echocardiographic evaluation of cardiac function in paediatric oncology patients treated with or without anthracycline. *Int J Cardiol* 1997;60:239-248.
- 267 Yeung ST, Yoong C, Spink J, Galbraith A, Smith PJ. Functional myocardial impairment in children treated with anthracyclines for cancer. *Lancet* 1991;337:816-818.
- 268 Weesner KM, Bledsoe M, Chauvenet A, Wofford M. Exercise echocardiography in the detection of anthracycline cardiotoxicity. *Cancer* 1991;68:435-438.
- 269 Klewer SE, Goldberg SJ, Donnerstein RL, Berg RA, Hutter JJ, Jr. Dobutamine stress echocardiography: a sensitive indicator of diminished myocardial function in asymptomatic doxorubicin-treated long-term survivors of childhood cancer. *J Am Coll Cardiol* 1992;19:394-401.
- 270 Ponikowski P, Anker SD, Chua TP, Szelemez R, Piepoli M, Adamopoulos S et al. Depressed heart rate variability as an independent predictor of death in chronic congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol* 1997;79:1645-1650.
- 271 Yi G, Goldman JH, Keeling PJ, Reardon M, McKenna WJ, Malik M. Heart rate variability in idiopathic dilated cardiomyopathy: relation to disease severity and prognosis. *Heart* 1997;77:108-114.
- 272 Fauchier L, Babuty D, Cosnay P, Autret ML, Fauchier JP. Heart rate variability in idiopathic dilated cardiomyopathy: characteristics and prognostic value. *J Am Coll Cardiol* 1997;30:1009-1014.
- 273 Jiang W, Hathaway WR, McNulty S, Larsen RL, Hansley KL, Zhang Y et al. Ability of heart rate variability to predict prognosis in patients with advanced congestive heart failure. *Am J Cardiol* 1997;80:808-811.
- 274 Brouwer J, van Veldhuisen DJ, Man in 't Veld AJ, Haaksma J, Dijk WA, Visser KR et al. Prognostic value of heart rate variability during long-term follow-up in patients with mild to moderate heart failure. The Dutch Ibopamine Multicenter Trial Study Group. *J Am Coll Cardiol* 1996;28:1183-1189.
- 275 Benedict CR, Weiner DH, Johnstone DE, Bourassa MG, Ghali JK, Nicklas J et al. Comparative neurohormonal responses in patients with preserved and impaired left ventricular ejection fraction: results of the Studies of Left Ventricular Dysfunction (SOLVD) Registry. The SOLVD Investigators. *J Am Coll Cardiol* 1993;22:146A-153A.
- 276 Bauch M, Ester A, Kimura B, Victorica BE, Kedar A, Phillips ML. Atrial natriuretic peptide as a marker for doxorubicin-induced cardiotoxic effects. *Cancer* 1992;69:1492-1497.
- 277 Yamashita J, Ogawa M, Shirakusa T. Plasma endothelin-1 as a marker for doxorubicin cardiotoxicity. *Int J Cancer* 1995;62:542-547.
- 278 Tikanoja T, Riikonen P, Perkkio M, Helenius T. Serum N-terminal atrial natriuretic peptide (NT-ANP) in the cardiac follow-up in children with cancer. *Med Pediatr Oncol* 1998;31:73-78.

- 279 Suzuki T, Hayashi D, Yamazaki T, Mizuno T, Kanda Y, Komuro I et al. Elevated B-type natriuretic peptide levels after anthracycline administration. *Am Heart J* 1998;136:362-363.
- 280 O'Brien PJ, Dameron GW, Beck ML, Kang YJ, Erickson BK, Di Battista TH et al. Cardiac troponin T is a sensitive, specific biomarker of cardiac injury in laboratory animals. *Lab Anim Sci* 1997;47:486-495.
- 281 Lipshultz SE, Rifai N, Sallan SE, Lipsitz SR, Dalton V, Sacks DB et al. Predictive value of cardiac troponin T in pediatric patients at risk for myocardial injury. *Circulation* 1997;96:2641-2648.
- 282 Missov E, Calzolari C, Davy JM, Leclercq F, Rossi M, Pau B. Cardiac troponin I in patients with hematologic malignancies. *Coron Artery Dis* 1997;8:537-541.
- 283 Wei CM, Lerman A, Rodeheffer RJ, McGregor CG, Brandt RR, Wright S et al. Endothelin in human congestive heart failure. *Circulation* 1994;89:1580-1586.
- 284 Hiroe M, Ohta Y, Fujita N, Nagata M, Toyozaki T, Kusakabe K et al. Myocardial uptake of ¹¹¹In monoclonal antimyosin Fab in detecting doxorubicin cardiotoxicity in rats. Morphological and hemodynamic findings. *Circulation* 1992;86:1965-1972.
- 285 Carrio I, Estorch M, Berna L, Lopez-Pousa J, Taberner J, Torres G. Indium-111-antimyosin and iodine-123-MIBG studies in early assessment of doxorubicin cardiotoxicity. *J Nucl Med* 1995;36:2044-2049.
- 286 Estorch M, Carrio I, Martinez-Duncker D, Berna L, Torres G, Alonso C et al. Myocyte cell damage after administration of doxorubicin or mitoxantrone in breast cancer patients assessed by indium 111 antimyosin monoclonal antibody studies. *J Clin Oncol* 1993;11:1264-1268.
- 287 Carrio I, Estorch M, Berna L, Germa JR, Alonso C, Ojeda B et al. Assessment of anthracycline-induced myocardial damage by quantitative indium 111 myosin-specific monoclonal antibody studies. *Eur J Nucl Med* 1991;18:806-812.
- 288 Narula J, Strauss HW, Khaw BA. Antimyosin positivity in doxorubicin cardiotoxicity: earlier than the conventional evidence. *J Nucl Med* 1993;34:1507-1509.
- 289 Kremer LCM, Tiel-van Buul MMC, Ubbink MC, M.Offringa J, Ottenkamp R, Valdes Olmos R et al. Indium-111-antimyosin scintigraphy in the early detection of heart damage after anthracycline therapy in children. *J Clin Oncol* 1999;17:1208-1211.
- 290 Carrio I, Estorch M, Lopez-Pousa A. Assessing anthracycline cardiotoxicity in the 1990s. *Eur J Nucl Med* 1996;23:359-364.
- 291 Sisson JC, Wieland DM, Sherman P, Mangner TJ, Tobes MC, Jacques SJ. Metaiodobenzylguanidine as an index of the adrenergic nervous system integrity and function. *J Nucl Med* 1987;28:1620-1624.
- 292 Imamura Y, Ando H, Mitsuoka W, Egashira S, Masaki H, Ashihara T et al. Iodine-123 metaiodobenzylguanidine images reflect intense myocardial adrenergic nervous activity in congestive heart failure independent of underlying cause. *J Am Coll Cardiol* 1995;26:1594-1599.
- 293 Henderson EB, Kahn JK, Corbett JR, Jansen DE, Pippin JJ, Kulkarni P et al. Abnormal I-123 metaiodobenzylguanidine myocardial washout and distribution may reflect myocardial adrenergic derangement in patients with congestive cardiomyopathy. *Circulation* 1988;78:1192-1199.
- 294 Wakasugi S, Fischman AJ, Babich JW, Aretz HT, Callahan RJ, Nakaki M et al. Metaiodobenzylguanidine: evaluation of its potential as a tracer for monitoring doxorubicin cardiomyopathy. *J Nucl Med* 1993;34:1283-1286.
- 295 Valdes Olmos RA, ten Bokkel Huinink WW, ten Hoeve RF, van Tinteren H, Bruning PF, van Vlies B et al. Assessment of anthracycline-related myocardial adrenergic derangement by [¹²³I]metaiodobenzylguanidine scintigraphy. *Eur J Cancer* 1995;31A:26-31.
- 296 Valdes Olmos RA, ten Bokkel Huinink WW, Dewit LG, Hoefnagel CA, Liem IH, van Tinteren H. Iodine-123 metaiodobenzylguanidine in the assessment of late cardiac effects from cancer therapy. *Eur J Nucl Med* 1996;23:453-458.
- 297 Takeishi Y, Sukekawa H, Sakurai T, Saito H, Nishimura S, Shibu T et al. Noninvasive identification of anthracycline cardiotoxicity: comparison of ¹²³I-MIBG and ¹²³I-BMIPP imaging. *Ann Nucl Med* 1994;8:177-182.
- 298 Bristow MR, Mason JW, Billingham ME, Daniels JR. Dose-effect and structure-function relationships in doxorubicin cardiomyopathy. *Am Heart J* 1981;102:709-718.
- 299 Bristow MR, Mason JW, Billingham ME, Daniels JR. Doxorubicin cardiomyopathy: evaluation by phonocardiography, endomyocardial biopsy, and cardiac catheterization. *Ann Intern Med* 1978;88:168-175.
- 300 Friedman MA, Bozdech MJ, Billingham ME, Rider AK. Doxorubicin cardiotoxicity. Serial endomyocardial biopsies and systolic time intervals. *JAMA* 1978;240:1603-1606.
- 301 Mason JW, Bristow MR, Billingham ME, Daniels JR. Invasive and noninvasive methods of assessing adriamycin cardiotoxic effects in man: superiority of histopathologic assessment using endomyocardial biopsy. *Cancer Treat Rep* 1978;62:857-864.

- 302 Bristow MR, Lopez MB, Mason JW, Billingham ME, Winchester MA. Efficacy and cost of cardiac monitoring in patients receiving doxorubicin. *Cancer* 1982;50:32-41.
- 303 Swain SM. Adult multicenter trials using dexrazoxane to protect against cardiac toxicity. *Semin Oncol* 1998;25:43-47.
- 304 Stuart NSA, Woodroffe CM, Grundy R, Cullen MH. Long-term toxicity of chemotherapy for testicular cancer-the cost of cure. *Br J Cancer* 1990;61:479-484.
- 305 Volksgezondheid Toekomst Verkenning 1997. De gezondheidstoestand: een actualisering. Bilthoven, The Netherlands: National Institute of Public Health and Environmental Protection. Maarssen, the Netherlands, Elsevier/De Tijdstroom, 1997.
- 306 de Wit R, Stoter G, Sleijfer DTh, Kaye SB, de Mulder PHM, ten Bokkel H et al. Four cycles of BEP versus an alternating regime of PVB and BEP in patients with poor-prognosis metastatic testicular non-seminoma; a randomised study of the EORTC Genitourinary Tract Cancer Cooperative Group. *Br J Cancer* 1995;71:1311-1314.
- 307 de Wit R, Stoter G, Sleijfer DTh, Neijt JP, ten Bokkel H, de Pricjck L et al. Four cycles of BEP vs four cycles of VIP in patients with intermediate- prognosis metastatic testicular non-seminoma: a randomized study of the EORTC Genitourinary Tract Cancer Cooperative Group. European Organization for Research and Treatment of Cancer. *Br J Cancer* 1998;78:828-832.
- 308 Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. Increasing prevalence of overweight among US adults. The National Health and Nutrition Examination Surveys, 1960 to 1991. *JAMA* 1994;272:205-211.
- 309 Schlant RC, Blomqvist CG, Brandenburg RO, DeBusk R, Ellestad MH, Fletcher GF et al. Guidelines for exercise testing. A report of the Joint American College of Cardiology/American Heart Association Task Force on Assessment of Cardiovascular Procedures (Subcommittee on Exercise Testing). *Circulation* 1986;74:653A-667A.
- 310 Berman DS, Kiat H, Maddahi J. The new 99mTc myocardial perfusion imaging agents: 99mTc-sestamibi and 99mTc-teboroxime. *Circulation* 1991;84:17-21.
- 311 Badano L, Stoian J, Cervasato E, Bosimini E, Gentile F, Giannuzzi P et al. Reproducibility of wall motion score and its correlation with left ventricular ejection fraction in patients with acute myocardial infarction. *Am J Cardiol* 1996;78:855-858.
- 312 Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH. Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. *Ann Intern Med* 1991;114:345-352.
- 313 Albers JJ, Warnick GR, Wiebe D, King P, Steiner P, Smith L et al. Multi-laboratory comparison of three heparin-Mn2+ precipitation procedures for estimating cholesterol in high-density lipoprotein. *Clin Chem* 1978;24:853-856.
- 314 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
- 315 National Cholesterol Education Program. Second Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). *Circulation* 1994;89:1333-1445.
- 316 Parving HH. Microalbuminuria in essential hypertension and diabetes mellitus. *J Hypertens Suppl* 1996;14:S89-S93.
- 317 Caro JF. Clinical review 26: Insulin resistance in obese and nonobese man. *J Clin Endocrinol Metab* 1991;73:691-695.
- 318 Pearson ES, Hartley H.O. Biometrika Tables for Statisticians (ed 3). London, UK, Biometrika Trust, 1976.
- 319 Einhorn LH, Williams SD, Loehrer PJ, Birch R, Drasga R, Omura G et al. Evaluation of optimal duration of chemotherapy in favorable-prognosis disseminated germ cell tumors: a Southeastern Cancer Study Group protocol. *J Clin Oncol* 1989;7:387-391.
- 320 Levi JA, Thomson D, Sandeman T, Tattersall M, Raghavan D, Byrne M et al. A prospective study of cisplatin-based combination chemotherapy in advanced germ cell malignancy: role of maintenance and long-term follow-up. *J Clin Oncol* 1988;6:1154-1160.
- 321 Samson MK, Rivkin SE, Jones SE, Costanzi JJ, LoBuglio AF, Stephens RL et al. Dose-response and dose-survival advantage for high versus low-dose cisplatin combined with vinblastine and bleomycin in disseminated testicular cancer. A Southwest Oncology Group study. *Cancer* 1984;53:1029-1035.
- 322 Stoter G, Sleijfer DTh, Bokkel Huinink WW, Kaye SB, Jones WG, van Oosterom AT et al. High-dose versus low-dose vinblastine in cisplatin-vinblastine- bleomycin combination chemotherapy of non-seminomatous testicular cancer: a randomized study of the EORTC Genitourinary Tract Cancer Cooperative Group. *J Clin Oncol* 1986;4:1199-1206.

- 323 Bosl GJ, Gluckman R, Geller NL, Golbey RB, Whitmore WF, Jr., Herr H et al. VAB-6: an effective chemotherapy regimen for patients with germ-cell tumors. *J Clin Oncol* 1986;4:1493-1499.
- 324 Bosl GJ, Geller NL, Vogelzang NJ, Carey R, Auman J, Whitmore WF et al. Alternating cycles of etoposide plus cisplatin and VAB-6 in the treatment of poor-risk patients with germ cell tumors. *J Clin Oncol* 1987;5:436-440.
- 325 Reynolds TF, Vugrin D, Cvitkovic E, Cheng E, Braun DWJ, O'Hehir MA et al. VAB-3 combination chemotherapy of metastatic testicular cancer. *Cancer* 1981;48:888-898.
- 326 Logothetis CJ, Samuels ML, Selig DE, Ogden S, Dexeus F, Swanson D et al. Cyclic chemotherapy with cyclophosphamide, doxorubicin, and cisplatin plus vinblastine and bleomycin in advanced germinal tumors. Results with 100 patients. *Am J Med* 1986;81:219-228.
- 327 Nichols CR, Williams SD, Loehrer PJ, Greco FA, Crawford ED, Weetlauffer J et al. Randomized study of cisplatin dose intensity in poor-risk germ cell tumors: a Southeastern Cancer Study Group and Southwest Oncology Group protocol. *J Clin Oncol* 1991;9:1163-1172.
- 328 Sagie A, Benjamin EJ, Galderisi M, Larson MG, Evans JC, Fuller DL et al. Echocardiographic assessment of left ventricular structure and diastolic filling in elderly subjects with borderline isolated systolic hypertension (the Framingham Heart Study). *Am J Cardiol* 1993;72:662-665.
- 329 Vanoverschelde JL, Wijns W, Michel X, Cosyns J, Detry JM. Asynchronous (segmental early) relaxation impairs left ventricular filling in patients with coronary artery disease and normal systolic function. *J Am Coll Cardiol* 1991;18:1251-1258.
- 330 Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990;322:1561-1566.
- 331 Ghali JK, Liao Y, Simmons B, Castaner A, Cao G, Cooper RS. The prognostic role of left ventricular hypertrophy in patients with or without coronary artery disease. *Ann Intern Med* 1992;117:831-836.
- 332 Damsgaard EM, Froland A, Jorgensen OD, Mogensen CE. Microalbuminuria as predictor of increased mortality in elderly people. *BMJ* 1990;300:297-300.
- 333 Travis LB, Curtis RE, Storm H, Hall P, Holowaty E, van Leeuwen FE et al. Risk of second malignant neoplasms among long-term survivors of testicular cancer. *J Natl Cancer Inst* 1997;89:1429-1439.
- 334 Messerschmidt J, Alt F, Tolg G, Angerer J, Schaller KH. Adsorptive voltametric procedure for the determination of platinum baseline levels in human body fluids. *Fresenius J Anal Chem* 1992;343:391-394.
- 335 Weisinger JR, Bellorin-Font E. Magnesium and phosphorus. *Lancet* 1998;352:391-396.
- 336 Cox IM, Campbell MJ, Dowson D. Red blood cell magnesium and chronic fatigue syndrome. *Lancet* 1991;337:757-760.
- 337 Sutton RA, Dirks JH. Disturbances of calcium and magnesium metabolism. In: Brenner BM, ed. *The Kidney*. Philadelphia: Saunders, 1996: 1038-85.
- 338 Tomiak E, Piccart M, Mignolet F, Sahnoud T, Paridaens R, Nooy M et al. Characterisation of complete responders to combination chemotherapy for advanced breast cancer: a retrospective EORTC Breast Group study. *Eur J Cancer* 1996;32A:1876-1887.
- 339 Bonadonna G, Zambetti M, Valagussa P. Sequential or alternating doxorubicin and CMF regimens in breast cancer with more than three positive nodes. Ten-year results. *JAMA* 1995;273:542-547.
- 340 Adjuvant systemic therapy for women with node-negative breast cancer. The Steering Committee on Clinical Practice Guidelines for the Care and Treatment of Breast Cancer. *CMAJ* 1998;158 Suppl 3:S43-S51.
- 341 Fisher B, Dignam J, Wolmark N, DeCillis A, Emir B, Wickerham DL et al. Tamoxifen and chemotherapy for lymph node-negative, estrogen receptor-positive breast cancer. *J Natl Cancer Inst* 1997;89:1673-1682.
- 342 Binkley PF, Nunziata E, Haas GJ, Nelson SD, Cody RJ. Parasympathetic withdrawal is an integral component of autonomic imbalance in congestive heart failure: demonstration in human subjects and verification in a paced canine model of ventricular failure. *J Am Coll Cardiol* 1991;18:464-472.
- 343 Ishise H, Asanoi H, Ishizaka S, Joho S, Kameyama T, Umeno K et al. Time course of sympathovagal imbalance and left ventricular dysfunction in conscious dogs with heart failure. *J Appl Physiol* 1998;84:1234-1241.
- 344 Eaton GM, Cody RJ, Nunziata E, Binkley PF. Early left ventricular dysfunction elicits activation of sympathetic drive and attenuation of parasympathetic tone in the paced canine model of congestive heart failure. *Circulation* 1995;92:555-561.
- 345 Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 1981;213:220-222.
- 346 de Vries EGE, ten Vergert EM, Mastenbroek CG, Dalesio O, Rodenhuis S. Breast cancer studies in The Netherlands [letter]. *Lancet* 1996;348:407-408.
- 347 de Graaf H, Dolsma WV, Willemse PHB, van der Graaf WTA, Sleijfer DTh, de Vries EGE et al. Cardiotoxicity from intensive chemotherapy combined with radiotherapy in breast cancer. *Br J Cancer* 1997;76:943-945.

- 348 Spain MG, Smith MD, Grayburn PA, Harlamert EA, DeMaria AN. Quantitative assessment of mitral regurgitation by Doppler color flow imaging: angiographic and hemodynamic correlations. *J Am Coll Cardiol* 1989;13:585-590.
- 349 Perry GJ, Helmcke F, Nanda NC, Byard C, Soto B. Evaluation of aortic insufficiency by Doppler color flow mapping. *J Am Coll Cardiol* 1987;9:952-959.
- 350 Pieper EP, Hellemans IM, Hamer HP, Ravelli AC, Jaarsma W, Breekland A et al. Biplane transesophageal color-flow Doppler imaging in assessing severity of mitral regurgitation: influence of hemodynamic circumstances and mechanism of regurgitation. *J Cardiothorac Vasc Anesth* 1996;10:748-755.
- 351 Weyman AE. Principles and practice of echocardiography, 2nd ed. Philadelphia, 1994:1289-1295.
- 352 Taylor R, Waggoner AD. Doppler assessment of left ventricular diastolic function: a review. *J Am Soc Echocardiogr* 1992;5:603-612.
- 353 Tuininga YS, Crijns HJGM, Brouwer J, van den Berg MP, Man in't Veld AJ, Mulder G et al. Evaluation of importance of central effects of atenolol and metoprolol measured by heart rate variability during mental performance tasks, physical exercise, and daily life in stable postinfarct patients. *Circulation* 1995;92:3415-3423.
- 354 Grassi G, Vailati S, Bertinieri G, Seravalle G, Stella ML, Dell'Oro R et al. Heart rate as marker of sympathetic activity. *J Hypertens* 1998;16:1635-1639.
- 355 The criteria of the New York Heart Association. In: Nomenclature and Criteria for diagnosis. 9th ed. Boston, Little, Brown, 1994.
- 356 Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996;93:1043-1065.
- 357 Erselcan T, Kairemo KJ, Wiklund TA, Hernberg M, Blomqvist CP, Tenhunen M et al. Subclinical cardiotoxicity following adjuvant dose-escalated FEC, high-dose chemotherapy, or CMF in breast cancer. *Br J Cancer* 2000;82:777-781.
- 358 Nixon AJ, Manola J, Gelman R, Bornstein B, Abner A, Hetelekidis S et al. No long-term increase in cardiac-related mortality after breast-conserving surgery and radiation therapy using modern techniques. *J Clin Oncol* 1998;16:1374-1379.
- 359 Dardir MD, Ferrans VJ, Mikhael YS, el Grindy MS, el Aasar AB, el Zawahry HM et al. Cardiac morphologic and functional changes induced by epirubicin chemotherapy. *J Clin Oncol* 1989;7:947-958.
- 360 Franciosa JA, Park M, Levine TB. Lack of correlation between exercise capacity and indexes of resting left ventricular performance in heart failure. *Am J Cardiol* 1981;47:33-39.
- 361 Rihal CS, Nishimura RA, Hatle LK, Bailey KR, Tajik AJ. Systolic and diastolic dysfunction in patients with clinical diagnosis of dilated cardiomyopathy. Relation to symptoms and prognosis. *Circulation* 1994;90:2772-2779.
- 362 Vasani RS, Benjamin EJ, Levy D. Prevalence, clinical features and prognosis of diastolic heart failure: an epidemiologic perspective. *J Am Coll Cardiol* 1995;26:1565-1574.
- 363 Vasani RS, Benjamin EJ, Levy D. Congestive heart failure with normal left ventricular systolic function. Clinical approaches to the diagnosis and treatment of diastolic heart failure. *Arch Intern Med* 1996;156:146-157.
- 364 Lopez M, Vici P. European trials with dexrazoxane in amelioration of doxorubicin and epirubicin-induced cardiotoxicity. *Semin Oncol* 1998;25:55-60.
- 365 Brutsaert DL, Sys SU. Relaxation and diastole of the heart. *Physiol Rev* 1989;69:1228-1315.
- 366 Ekholm EM, Salminen EK, Huikuri HV, Jalonen J, Antila KJ, Salmi TA et al. Impairment of heart rate variability during paclitaxel therapy. *Cancer* 2000;88:2149-2153.
- 367 Bennett JM, Muss HB, Doroshow JH, Wolff S, Krentz ET, Cartwright K et al. A randomized multicenter trial comparing mitoxantrone, cyclophosphamide, and fluorouracil with doxorubicin, cyclophosphamide, and fluorouracil in the therapy of metastatic breast carcinoma. *J Clin Oncol* 1988;6:1611-1620.
- 368 McDonagh TA, Robb SD, Murdoch DR, Morton JJ, Ford I, Morrison CE et al. Biochemical detection of left-ventricular systolic dysfunction. *Lancet* 1998;351:9-13.
- 369 Cottin Y, Touzery C, Dalloz F, Coudert B, Toubeau M, Riedinger A et al. Comparison of epirubicin and doxorubicin cardiotoxicity induced by low doses: evolution of the diastolic and systolic parameters studied by radionuclide angiography. *Clin Cardiol* 1998;21:665-670.
- 370 Lerman A, Gibbons RJ, Rodeheffer RJ, Bailey KR, McKinley LJ, Heublein DM et al. Circulating N-terminal atrial natriuretic peptide as a marker for symptomless left-ventricular dysfunction. *Lancet* 1993;341:1105-1109.
- 371 Dishman RK, Nakamura Y, Garcia ME, Thompson RW, Dunn AL, Blair SN. Heart rate variability, trait anxiety, and perceived stress among physically fit men and women. *Int J Psychophysiol* 2000;37:121-133.
- 372 Moss AJ. Measurement of the QT interval and the risk associated with QTc interval prolongation: a review. *Am J Cardiol* 1993;72:238-258.

- 373 Billingham ME, Bristow MR, Glatstein E, Mason JW, Masek MA, Daniels JR. Adriamycin cardiotoxicity: endomyocardial biopsy evidence of enhancement by irradiation. *Am J Surg Pathol* 1977;1:17-23.
- 374 Huddart RA, Norman A, Coward D, Nicholls E, Jay G, Shadidi M et al. The health of long term survivors of testicular cancer. *Proc Am Soc Clin Oncol* 2000; abstract 1301.
- 375 de Wit R, Roberts JT, Wilkinson P, de Mulder PHM, Mead GM, Fossa SD et al. Final Analysis Demonstrating the Equivalence of 3 BEP vs. 4 Cycles and the 5 Day Schedule vs. 3 Days Per Cycle in Good Prognosis Germ Cell Cancer. An EORTC/MRC Phase III Study. *Proc Am Soc Clin Oncol* 2000; abstract 1281.
- 376 Quyyumi AA. Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. *Am J Med* 1998;105:32S-39S.
- 377 Zgliczynski S, Ossowski M, Slowinska-Srzednicka J, Brzezinska A, Zgliczynski W, Soszynski P et al. Effect of testosterone replacement therapy on lipids and lipoproteins in hypogonadal and elderly men. *Atherosclerosis* 1996;121:35-43.
- 378 Hellmann K. Overview and historical development of dexrazoxane. *Semin Oncol* 1998;25:48-54.
- 379 Herman EH, Lipshultz SE, Rifai N, Zhang J, Papoian T, Yu ZX et al. Use of cardiac troponin T levels as an indicator of doxorubicin-induced cardiotoxicity. *Cancer Res* 1998;58:195-197.
- 380 Nakamura T, Ueda Y, Juan Y, Katsuda S, Takahashi H, Koh E. Fas-mediated apoptosis in adriamycin-induced cardiomyopathy in rats: In vivo study. *Circulation* 2000;102:572-578.
- 381 Hofstra L, Liem IH, Dumont EA, Boersma HH, van Heerde WL, Doevendans PA et al. Visualisation of cell death in vivo in patients with acute myocardial infarction. *Lancet* 2000;356:209-212.
- 382 Sparano JA. Doxorubicin/taxane combinations: cardiac toxicity and pharmacokinetics. *Semin Oncol* 1999;26:14-19.
- 383 Feldman AM, Lorell BH, Reis SE. Trastuzumab in the treatment of metastatic breast cancer: anticancer therapy versus cardiotoxicity. *Circulation* 2000;102:272-274.

DANKWOORD

Dit proefschrift is het resultaat van een vruchtbare samenwerking tussen de afdelingen Medische Oncologie en de Cardiologie van het Academisch Ziekenhuis Groningen. Veel mensen hebben de afgelopen jaren een belangrijke bijdrage geleverd aan dit promotieonderzoek. Als eerste wil ik het ijzersterke 'supportive care team' wat de afgelopen jaren om mij heen heeft gestaan bedanken, omdat het zonder hen zeker niet was gelukt.

Mijn eerste promotor prof. dr. D.Th. Sleijfer. Beste Dirk, ik ben je zeer erkentelijk voor de bijna vaderlijke wijze waarop je mij de afgelopen jaren hebt begeleid. Je orde-in-de-chaos-scheppend-vermogen, je kritische blik worden reeds decennia lang geroemd in dankwoorden van voorgangers promovendi, en ik sluit me graag bij hen aan. Je was altijd bereid je werkzaamheden te onderbreken voor een warrige vraag of een verzuchting van mijn kant. Ook je steun op de wat taaiere momenten van het promotieonderzoek, heb ik als bijzonder waardevol ervaren.

Mijn tweede promotor prof. dr. D.J. van Veldhuisen. Beste Dirk Jan, je enthousiasme voor je vak en voor het doen van onderzoek werkten vaak als een opkikker. Het is niet altijd even gemakkelijk om als cardioloog elke maand weer van die lastige oncologen op je kamer te krijgen, maar daar sloeg je je toch altijd prima doorheen. Ik vind het prachtig dat je mijn promotor hebt kunnen worden, en dank je hartelijk voor de belangrijke bijdrage die je hebt geleverd aan dit promotieonderzoek.

Mijn co-promotor Dr. W.T.A. van der Graaf. Beste Winette, ik vond het erg prettig met jou te mogen samenwerken. Je frisse kijk op onderzoeksresultaten en je nauwkeurige werkwijze hebben veel aan de kwaliteit van dit proefschrift bijgedragen.

Mijn referent Dr. J.A. Gietema. Beste Jourik, een betere coach had ik mij niet kunnen wensen. Je vormde de perfecte schakel tussen mij en 'de bazen' waar je inmiddels ook zelf toe behoort. Met opbeurende opmerkingen als "strijder", "het kan altijd erger" en "leven is lijden", wist je me door dipjes heen te slepen. Ik waardeer je zeer om je efficiënte werkwijze, je scherp analyserend vermogen, je abonnement op Lancet letters, maar vooral als persoon.

Prof. dr. E.G.E. de Vries. Beste Liesbeth, ondanks dat je bij vele tientallen projecten bent betrokken, heb je je ook weer voor dit promotieonderzoek volop ingezet, waarvoor hartelijke dank. Hoe je dat allemaal doet, blijft een raadsel.

Verder wil ik alle medeauteurs bedanken voor hun inbreng bij de totstandkoming van de diverse hoofdstukken. Dr. Maarten van den Berg wil ik bedanken voor zijn bijdrage in met name het echocardiografische deel van het onderzoek en dr. Jaap Haaksma voor zijn hulp bij de analyse en interpretatie van de HRV resultaten. Voor de statistiek heb ik veel gehad aan Dr. Wim Sluiter en drs. Corine Volkers. Dr. Frans Boomsma, was verantwoordelijk voor de ANP en BNP bepalingen en veel leuke gesprekken over de telefoon. Dr. D.A. Piers en Dr. P.L. Jager van de afdeling nucleaire geneeskunde dank ik voor de behoordeling van de vele MUGA-scans. Diverse hoofdstukken werden op deskundige wijze van commentaar voorzien door Prof.dr. H. Schraffordt Koops, dr. P.H.B. Willemse, prof. dr. N.H. Mulder, en dr. W.V. Dolsma. Prof. Dr. D.R.A. Uges dank ik voor zijn bijdrage aan de hoofdstukken 5 en 6.

Drs. Michael Schaapveld van het IKN dank ik voor zijn statistische hulp bij hoofdstuk 4. Ronald Gijssen van het RIVM was erg behulpzaam met het beschikbaar stellen van de incidentiecijfers van hart- en vaatziekten van de algemene Nederlandse bevolking. Als student hebben drs. Myrthe Runne, drs. Tom Gelever, en drs. Anne Rixt Buursma een essentiële bijdrage geleverd aan respectievelijk de hoofdstukken 4, 5 en 6.

Alle regas-medewerkers en dokters-assistenten van de polikliniek interne geneeskunde, als ook van het functiecentrum cardiologie waren onmisbaar voor de logistieke ondersteuning.

Willy Bruins dank ik voor het zorgvuldig opleuken van diverse manuscripten en alle verdere secretariële ondersteuning.

Alle patiënten die voor dit proefschrift belangeloos 24-uurs urine hebben verzameld of met vervelende 'kastjes' hebben moeten rondlopen, dank ik voor hun medewerking.

De leden van de promotiecommissie Prof. Dr. H.J.G.M. Crijns, Prof. Dr. Ir. F.E. van Leeuwen en Prof. Dr. N.H. Mulder, dank ik voor de tijd en aandacht die zij hebben besteed aan de beoordeling van dit proefschrift.

Beste H0-ers, (ik hoop voor jullie dat dit toch het echt het laatste proefschrift is dat jullie zo kunnen worden genoemd) jullie zorgden voor de bijzondere werksfeer, alle gezelligheid, zin- en onzinpraat, te lange theepauzes, noodstroomborrels, of zomaar borrels die het leven als promovendus aanzienlijk veraangenaamen.

Mijn paranimfen Peter Kraaij (ex-collega microlight eigenaar) en Peter-Edward Boer (collega luidmeester) dank ik alvast voor de zware taak die zij op zich hebben genomen om mij praktisch en moreel te ondersteunen. Ik verheug me nu al op het moment dat wij onze pakjes aanmogen.

Familie en vrienden wil ik bedanken voor alle mentale support de afgelopen jaren. Tot slot, lieve Bouke en Nina, ontzettend bedankt voor alle kansen en steun die jullie mij de afgelopen 3 decennia, en ook weer tijdens dit promotieonderzoek hebben gegeven.

Martin Meinardi