Chapter 4

Chemotherapy for testicular cancer induces acute alterations in diastolic heart function

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ABSTRACT

Purpose: After treatment with cisplatin-based chemotherapy for testicular cancer (TC), patients have a higher prevalence of cardiovascular complications after long-term follow up. Little is known about acute cardiovascular effects of chemotherapy. Therefore, the purpose of the present study was to explore short-term effects of chemotherapy on cardiac function, using magnetic resonance (MR) techniques in patients treated for TC.

Methods: Fourteen TC patients (age 34.6 ± 12.3 years) were studied before and early after treatment with cisplatin-based chemotherapy. Assessment of cardiac function was performed with MRI. Serum glucose and insulin were measured after an overnight fast and insulin sensitivity, reflected by the Quicki index, was calculated.

Results: At baseline and 3 months after start of the chemotherapy patients underwent MRI examination. Left ventricular (LV) end diastolic volume and LV stroke volume significantly decreased from 192.3 ± 26.8 ml to 174.7 ± 25.7 ml ($p<0.05$) and 108.8 ± 17.6 ml to 94.7 ± 16.3 ml ($p<0.05$) respectively. The ratio of early (E) and atrial (A) filling velocities across the mitral valve (E/A ratio), a parametre of diastolic heart function, was significantly decreased after chemotherapy from 1.87 ± 0.43 to 1.64 ± 0.45, $p<0.01$. After chemotherapy metabolic parameters were unfavorably changed, reflected by a significantly decreased Quicki index, which reduced from 0.39 ± 0.05 to 0.36 ± 0.05 ($p<0.05$).

Conclusions: Chemotherapy for testicular cancer induces acute alterations in diastolic heart function, paralleled by unfavorable metabolic changes. Therefore, early after chemotherapy, metabolic treatment may be indicated to possibly reduce long-term cardiovascular complications.

INTRODUCTION

Testicular cancer (TC) is the most frequent form of cancer in young men. The prognosis of TC is good, with high cure rates since the introduction of treatment with cisplatin-based chemotherapy.[74] Because of the increasing number of survivors with a long life expectancy, understanding and prevention of short-term and long-term cardiovascular effects of chemotoxicity is very important.

Treatment of TC with cisplatin, bleomycin and etoposide (BEP) combination chemotherapy is associated with acute vascular toxicity and subacute changes in cardiac function [98;117], as well as with long-term cardiovascular disease.[28;29;47] Previous studies showed that cisplatin and bleomycin induce alterations in endothelial function and endothelial damage *in vitro*.[62;63] These findings suggest direct toxic effects of chemotherapy on the cardiovascular system. Little is known about acute effects of cisplatin-based chemotherapy on cardiac function. More insight in the pathophysiology of the direct toxic effect of cisplatin-based chemotherapy on cardiac function and vessel wall is relevant to possibly prevent long-term cardiovascular disease. One previous study reported sub-acute deterioration of diastolic function, assessed with echocardiography ten months after cisplatin-based chemotherapy.[117]

Indirect effects of chemotherapy also seem to play a role in the increased risk of cardiovascular complications. For example, early after treatment with cisplatin-based chemotherapy changes in serum lipids have been described.[117] Additionally, higher incidences of hypercholesterolemia, hypertension, microalbuminuria, obesity, elevated insulin-glucose ratio, and thereby metabolic syndrome have been reported at least three years after chemotherapy.[118-120] The acute effects of chemotherapy, defined as effects occurring three months after start of chemotherapy, on these risk factors are largely unknown. The aforementioned indirect risk factors are all independently associated with a higher risk of cardiovascular disease and may contribute to the overall increased risk of cardiovascular complications after treatment with cisplatin-based chemotherapy. The increased risk of cardiovascular disease in cured TC patients after cisplatin-based chemotherapy is probably a combination of direct and indirect effects of chemotherapy.[74;120] Early changes in cardiac function and risk factors may have prognostic value for long-term development of cardiovascular complications.[121] Magnetic resonance (MR) imaging is a highly reproducible imaging modality to assess cardiac function. Furthermore, myocardial triglyceride (TGC) content can be measured with proton magnetic resonance spectroscopy (^{1}H-MRS).[122] Additionally, abdominal visceral and subcutaneous fat volume can be accurately assessed with MRI.[123] Therefore, the purpose of this study was to investigate acute

changes in cardiac function and myocardial TGC, in relation to body fat distribution and metabolic parameters three months after start with chemotherapy for TC, assessed with MR-techniques.

MATERIALS AND METHODS

This study was approved by the local medical ethics committee and all subjects gave written informed consent. Metastatic TC patients, scheduled for first-line curative cisplatin-based combination chemotherapy in the Leiden University Medical Center were included between 2007 and 2009. Exclusion criteria were co-morbidities, including cardiovascular disease and diabetes mellitus.

Patients received three or four cycles of standard BEP-chemotherapy repeated every 3 weeks. Each cycle consisted of intravenously administered etoposide (100mg/m^2 over 1 hr, days 1-5), cisplatin (20mg/m^2 over 4 hr, days 1-5), and bleomycin (30 IUSP over 30min) at days 2, 8, and 15. According to Dutch oncological guidelines, TC patients with good prognosis were treated with three cycles of BEP and patients with intermediate prognosis were treated with four cycles BEP. One patient, in addition received paclitaxel (175mg/m^2) on day 1 of each of his 4 chemotherapy cycles as part of a randomized phase III study comparing paclitaxel-BEP and standard BEP in patients with intermediate prognosis TC. All patients were orchidectomized before adjuvant chemotherapeutic treatment.

BMI was determined at baseline and after chemotherapy. Fasting serum glucose, insulin, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides were determined. Insulin resistance was assessed with the quantitative insulin sensitivity index (Quicki index), which is calculated using the formula: 1 / (log(fasting insulin μU/mL) + log(fasting glucose mg/dL))**.**[124] Renal function defined as estimated glomerular filtration rate was calculated with the Modification of Diet in Renal Disease (MDRD) equation: 186 * (serum creatinine μmol/L / 88,4)$^{-1.154}$ x (age)$^{-0.203}$.

Patients underwent MRI before start and shortly after the last chemotherapy cycle, which was approximately three months after start of chemotherapy. Blood pressure and heart rate were measured during MRI using a semiautomated sphygmomanometre (Dinamap, Critikon, Tampa, Fla, USA). We have included part of a studygroup from a previous study, describing metabolic changes and MRI assessment of hepatic triglyceride content, aortic pulse wave velocity and abdominal fat mass in TC patients undergoing curative chemotherapy at 3 and 9 months after start of chemotherapy.[125]

MRI protocol

Left and right ventricular function

Cardiac imaging was performed using a 1.5 Tesla whole-body MRI scanner (Gyroscan ACS-NT15; Philips Medical Systems, Best, The Netherlands) after a night fast. The heart was imaged in short-axis orientation, using electrocardiographically gated breath-hold cine steady-state free-precession sequences as previously described.[126] Imaging parameters were: repetition time (TR) 3.4ms, echo time (TE) 1.7ms, flip angle (FA) 35º, field of view (FOV) 400×320mm, slice thickness 10mm, no slice gap was used. To assess LV and RV systolic function, endocardial contours were manually drawn, using MASS® software (Medis, Leiden, The Netherlands). LV and RV ejection fraction (EF), stroke volume (SV), end-diastolic volume (EDV) and end-systolic volume (ESV) were assessed. Epicardial contours of the LV were drawn to calculate LV end-diastolic mass (LVED mass).

To assess LV and RV diastolic function, flow across mitral and tricuspid valve was measured using an electrocardiographically gated gradient echo sequence with velocity-encoding. Scan parameters: TR=9.1ms, TE=1.0ms, FA=20º, slice thickness=8mm, FOV=350mm^2 and matrix=256x256 pixels. Flow velocities in early diastole (E) and at atrial contraction (A) were measured and peak flow ratio was calculated (E/A ratio) using FLOW® software (Medis, Leiden, The Netherlands). The downslope of the early filling phase (E deceleration peak) and LV filling pressures (E/Ea) were calculated.[127;128]

Myocardial triglyceride content

Myocardial ^{1}H-MR spectra were obtained as described before.[122] A voxel was positioned in the myocardial interventricular septum in end-systole. An ECG triggering and a respiratory pencil beam navigator were used during acquisition. Spectra with water suppression were acquired with TE=26ms and TR≥3,000ms. 1,024 data points were collected using a 1,000-Hz spectral width and averaged over 128 acquisitions. Spectra without water suppression with TR=10s and four averages were obtained without changing other parameters. Spectroscopic data were fitted using validated software (jMRUI version 2.2, Leuven, Belgium).[129] Myocardial TGC content was calculated as (amplitude of TGC signal/amplitude water signal) x 100%.

Pericardial fat

Pericardial fat was quantified as described previously using electrocardiographically gated breath-holds with balanced turbo-field echo MR sequence.[130] Imaging parameters: TR=3.2ms, TE=1.60ms, FA=50°, slice thickness=10mm, FOV=400mm^2. The four-chamber view was analysed, with the plane of respiratory mitral and tricuspid valves as margins. To quantify periventricular fat volume, contours around pericardial fat were drawn manually at end-systole and multiplied by the thickness of the slice. We used MASS® for postprocessing.

Visceral and subcutaneous fat

Visceral and subcutaneous fat volumes were imaged using a turbo spin echo imaging sequence.[123] During one breath-hold, three consecutive transversal slices of 10mm thickness were scanned at the fifth lumbar vertebrae. Imaging parameters: TR=168ms, TE=11ms and FA=90º. Contours were drawn around visceral and subcutaneous abdominal fat depots using MASS®. Visceral and subcutaneous fat areas of each slice were multiplied by the slice thickness to acquire a volume and the volumes of all three slices were summed.

Statistical Analysis

Statistical analyses were performed using SPSS 17.0 (SPSS Inc.Chicago, Illinois, USA). We used two-tailed paired t-tests to compare the two study timepoints, since all data were normally distributed. To determine which significantly changed parameters influenced the differences of the other cardiac parameters, univariate regression analyses were performed. In these regression analyses the delta of the significantly changed parametre (the difference of the parametre before and after chemotherapy) was the independent variable and the delta of the cardiac parametre of interest was the dependent variable. In case of a significant influence the corrected difference between baseline and follow-up was extracted from the regression analysis. A *P*-value of < 0.05 was considered statistically significant. Data are expressed as mean±standard deviation (SD).

RESULTS

Forty consecutive patients were asked to participate. Twenty-one patients could not participate, based on logistic reasons (*N*=5), refusal or non-eligibility (*N*=16). These patients had unwillingness to undergo frequent blood drawings during chemotherapy or MRI. Nineteen patients underwent baseline MRI. Five patients missed the follow-up MRI due to treatment-related sickness (*N*=2), study withdrawal (*N*=2) and treatment-related death (*N*=1). Accordingly, 14 patients were included in data analysis of the present study. Three HDL-concentrations and 1 insulin-concentration were missing.

Four patients (28.6%) had a seminoma, two patients (14.3%) a non-seminoma and eight patients (57.1%) had a combined tumor. Seven patients (50%) had para-aortic lymph node metastasis (stage II) and the other half of the patients had distant metastasis (stage III). Table 1 shows the patient characteristics at baseline and after chemotherapy. Average age was 35±12 years. Average time between the two MRI scans was 2.6±0.5 months. Time between the last day of chemotherapy and the MRI after chemotherapy was 18±18 days. Weight, BMI and blood pressure did not change during follow-up. Heart rate increased significantly from 64±9 beats per minute to 76±15 beats per minute (p=0.007). Laboratory parameters at baseline and at follow-up are described in Table 2. The Quicki index decreased, from 0.39±0.05 to 0.36±0.05 (p=0.02), reflecting greater insulin resistance.

Table 1. Patient characteristics at baseline and after chemotherapy.

Parameters mean±SD	Baseline	After chemotherapy	p-value
Systolic blood pressure (mmHg)	123 ± 17	118 ± 11	NS
Diastolic blood pressure (mmHg)	73 ± 11	70 ± 12	NS
Heart rate (bpm)	64 ± 9	76 ± 15	0.007
Weight (kg)	83.3 ± 15.5	84.5 ± 18.5	NS
Body Mass Index (kg/m^2)	24.4 ± 4.0	24.7 ± 4.6	NS
Cholesterol (mmol/l)	4.7 ± 1.3	5.5 ± 1.5	< 0.01
Estimated GFR (ml/min)	102 ± 16	113 ± 18†	< 0.01
HDL (mmol/l)	1.30 ± 0.31	1.36 ± 0.25	NS
LDL (mmol/l)	3.12 ± 1.15	3.74 ± 1.41	< 0.05
Triglycerides (mmol/l)	1.16 ± 0.60	1.64 ± 1.11	NS
Fasting glucose (mmol/l)	5.1 ± 0.5	5.2 ± 0.6	NS
Insulin (mU/l)	6.2 ± 5.0	9.8 ± 6.8	NS
Quicki index	0.39 ± 0.05	0.36 ± 0.05	0.02

NS = not significant.

Left and right ventricular function

Due to technical difficulties two diastolic LV and RV scans and one systolic RV were missing. LV EDV and SV significantly decreased, respectively from 192±27ml to 175±26ml (p=0.01) and from 109±18ml to 95±16ml (p=0.03) (Table 3). Although LV EDV and SV were significantly influenced by the increased heart rate, the difference between baseline and follow-up remained significant after correction for heart rate. The other systolic LV parameters did not change after chemotherapy (Table 3).

LV E/A ratio decreased significantly after chemotherapy from 1.87±0.43 to 1.64±0.45 (p=0.009). In addition, the atrial peak filling rate increased significantly after chemotherapy. The LV E/A ratio and the atrial peak filling rate were not significantly influenced by the increased heart rate, thus correction for heart rate was not required. Other LV diastolic function parameters did not change after chemotherapy.

Right ventricular EDV and SV decreased significantly, from 210±32 ml to 196±32 ml and from 105±14 (p=0.01) to 93±16 (p=0.04) respectively. Both parameters were influenced by the increased heart rate. After correction for heart rate the difference between baseline and follow-up remained statistically significant. All other RV parameters, systolic or diastolic, remained unchanged after chemotherapy (Table 2).

Table 2. Parameters of myocardial function, assessed with MRI at baseline and after cisplatin-based chemotherapy in TC patients.

	Left Ventricle			**Right Ventricle**		
	Baseline	After chemotherapy	p-value	Baseline	After chemotherapy	p-value
Systolic function						
EDV (ml)	192 ± 27	175 ± 26	0.01	210 ± 32	196 ± 38	0.01
ESV (ml)	84 ± 16	80 ± 14	NS	105 ± 29	103 ± 25	NS
SV (ml)	109 ± 18	95 ± 16	0.03	105 ± 14	93 ± 16	0.04
CO (ml/min)	6816 ± 1112	7050 ± 1160	NS	6595 ± 927	6976 ± 851	NS
EF (%)	56.6 ± 5.3	54.3 ± 5.1	NS	49.9 ± 6.7	47.9 ± 4.6	NS
Diastolic function						
E peak filling rate (ml/s)	657 ± 119	662 ± 122	NS	415 ± 55	406 ± 60	NS
E deceleration (ml/s^2x10^{-3})	6.3 ± 2.2	6.0 ± 1.7	NS	3.7 ± 1.9	3.0 ± 1.2	NS
A peak filling rate (ml/s)	362 ± 78	425 ± 111	NS	311 ± 57	325 ± 100	NS
E/A-peak ratio	1.87 ± 0.43	1.64 ± 0.45	0.009	1.37 ± 0.25	1.32 ± 0.31	NS
E/Ea	6.8 ± 2.0	6.8 ± 2.0	NS	~	~	

NS = not significant.

Fat distribution

Baseline and follow-up data from eight myocardial ^{1}H-MR spectra were present. Myocardial triglyceride content did not significantly change after chemotherapy; at baseline TGC content was 0.69±0.41% and after chemotherapy TGC content was 0.74±0.35%. Pericardial fat volume did not change significantly after chemotherapy; 27.0±3.9ml at baseline and 28.1±5.5ml early after chemotherapy.

One waist fat MRI scan was missing. Visceral fat volume increased significantly from 186±125ml to 227±162ml (p=0.04), whereas subcutaneous fat volume did not change. The visceral/subcutaneous fat ratio did significantly increase from 0.38±0.11 to 0.42±0.12 (p=0.03).

DISCUSSION

The main finding of this study is that LV diastolic function is decreased 3 months after start of cisplatin-based chemotherapy for TC. Decreased diastolic function was accompanied by an unfavorable change in metabolic profile as measured by increased serum LDL and total cholesterol and decreased insulin sensitivity. Additionally, visceral fat volume and visceral/subcutaneous fat ratio increased. Several studies reported increased cardiovascular risk factors, increased incidence of cardiovascular disease and diminished cardiac function as long-term complications in TC patients within years after treatment with cisplatin-based chemotherapy.[27;29;121;131;132] Only few studies report on the (sub-)acute cardiovascular effects of cisplatin-based chemotherapy.[63;117;133] To the best of our knowledge we are the first to investigate the acute effects of chemotherapy on cardiac function. Altena et al. showed deterioration of diastolic heart function assessed with echocardiography approximately 10 months after chemotherapy.[117] In contrast, we assessed cardiac function immediately after completion of chemotherapy.

In the present study the LV E/A ratio decreased, reflecting deterioration in diastolic LV function. Since the E/A ratio is load dependent and thus influenced by the filling status of the patient, an estimation of LV filling pressure was determined (E/Ea) [128], which did not change after chemotherapy. Therefore, the decreased E/A ratio after chemotherapy presumably reflects disturbed intrinsic relaxation of the LV, rather than change in LV filling pressure. A previous study showed progressive deterioration of diastolic heart function, 10 months and 6.9 years after cisplatin-based chemotherapy [49]. Therefore, acute changes in diastolic function observed in the present study might be of prognostic clinical significance. Long-term follow-up data of our patient group would be interesting to have some information of the predictive value of these early

cardiac changes. LV ejection fraction (LVEF), an important parametre of systolic function, did not change. Change in LV diastolic function with preserved LVEF after treatment with cisplatin-based chemotherapy is in line with previous studies.[117;121;132] It is known that diastolic dysfunction precedes a decline in systolic function and can be regarded as an important prognostic marker of ongoing disease.[63;129] For future studies it would be interesting to combine echocardiography with cardiac MRI, since previous studies suggest that early impairment of systolic function may also be detected using strain echocardiography and that it could be predictive of subsequent reduction in LVEF.[134] Furthermore, in further studies biomarkers such as N-terminal pro-brain natriuretic peptide and troponin I could be determined, because determination of these biomarkers may be useful in evaluation of early cardiac toxicity.[134]

Cisplatin can directly injure cardiomyocytes through oxidative stress and mitochondrial damage.[135] Additionally, cisplatin and bleomycin cause decreased endothelial cell survival and induce apoptosis of endothelial cells *in vitro*.[63] These endothelial changes may promote inflammation and atherosclerosis, which can contribute to chemotherapy-induced vascular toxicity. In addition, endothelial cells at the endocardium play an obligatory role in maintaining cardiac function.[136] Cisplatin-based chemotherapy may also indirectly lead to cardiovascular disease, via increased prevalence of cardiovascular risk factors.[29;61] Increased prevalence of cardiovascular risk factors, such as dyslipidemia, central obesity and insulin resistance, can lead to accelerated atherosclerosis.[135] In this study the follow-up time is presumably too short for these indirect effects of chemotherapy to contribute to impaired cardiac function. We could not establish a direct relationship between cardiac function and metabolic profile. However, already 3 months after start of chemotherapy, we identify a shift to an unfavorable metabolic profile: visceral fat volume, visceral/subcutaneous fat ratio, LDL-cholesterol and total cholesterol were increased and insulin sensitivity decreased. Visceral fat is more deleterious than subcutaneous fat and is associated with the metabolic syndrome and cardiovascular disease.[61;137;138] The metabolic syndrome consists of a cluster of risk factors: dyslipidemia, hypertension, central obesity and insulin resistance. This syndrome is associated with a long-term increased risk for atherosclerotic disease [61;79], with cardiovascular disease as one of the major complications. Via insulin resistance and the concomitant increased release of adipokines such as resistin, the metabolic syndrome is associated with endothelial dysfunction.[139] High C-reactive protein (CRP) levels are associated with the metabolic syndrome and endothelial dysfunction.[120] In this study we did not measure CRP levels unfortunately, but in subsequent studies these levels should be measured. A recent

study showed that the metabolic syndrome is more prevalent and develops at earlier age in TC survivors, treated with cisplatin-based chemotherapy [49]. Visceral adipose tissue contributes to insulin resistance [138], which is associated with decreased cardiac function [118;140;141], even in absence of diabetes mellitus [119;142]. In the metabolic syndrome, insulin resistance and (visceral) adiposity is correlated with myocardial TGC accumulation, which might negatively influence cardiac function.[143;144] In this study we did not find a difference between myocardial TGC content before and after chemotherapy. The number of measurements of myocardial TGC (*N*=8) content is probably too small to draw firm conclusions regarding myocardial TGC changes early after chemotherapy. Another explanation could be that the follow-up period is too short, so the oxidative capacity of the myocardium is still sufficient, preventing storage of TGC in the myocardium.

Diastolic cardiac function progressively deteriorates in TC survivors treated with cisplatin-based chemotherapy.[121] Subclinical changes in cardiac diastolic function may therefore precede late clinical dysfunction. If these early changes are predictive for later abnormalities in cardiac function, such changes may be used to monitor patients more specifically. Furthermore, patients treated with cisplatin-based chemotherapy are at increased risk of developing an unfavorable cardiovascular-risk profile, which can contribute to development of long-term cardiac failure. Accordingly, early detection of risk factors for cardiovascular disease is important, as treatment of the unfavorable metabolic changes with lifestyle intervention or medication can contribute to an improved long-term prognosis in patients treated with cisplatin-based chemotherapy.

In conclusion, treatment with cisplatin-based chemotherapy for TC induces acute alterations in diastolic cardiac function, paralleled by unfavorable metabolic changes. Although the predictive significance of the diastolic cardiac changes for long-term cardiovascular morbidity is not clear at present, it seems plausible that they may eventually lead to overt cardiovascular disease. As the detrimental metabolic changes can contribute to the development of cardiovascular disease, these risk factors should be monitored and treated if necessary.