Chapter 11

Summary

Testicular cancer is a highly curable disease that mainly affects men between 20 and 40 years of age. Nowadays, over 80% of patients become long-term survivors following treatment with chemotherapy that consists of a combination of bleomycin, etoposide, and cisplatin (BEP-regimen). Unfortunately, vascular toxic effects of chemotherapy have been described in testicular cancer patients in both the short and the long term. These vascular complications may cause serious morbidity and sometimes mortality in survivors who are generally young. Moreover, vascular toxicity occurring during treatment may interfere with the continuation of a potentially curative treatment and thus reduce the chance of cure. It is largely unknown how and in which patients vascular toxicity will develop. However, this knowledge is needed for the development of appropriate prevention strategies and for the selection of high risk patients who are most likely to benefit from this.

In chapter 2, we described and discussed the development of a range of metabolic disorders in survivors of various malignancies, including testicular cancer, following multimodality cancer treatment. These metabolic disorders include dyslipidaemia, overweight, and insulin resistance, which are all components of the so-called metabolic syndrome. The metabolic syndrome is an important risk factor for the development of cardiovascular disease in the general population. An increased risk for cardiovascular disease has also been reported in long-term survivors of childhood malignancies and testicular cancer. Therefore, the development of the metabolic syndrome in survivors of cancer after multimodality treatment may be important. We described association studies in the general population demonstrating correlations between the components of the metabolic syndrome and hormonal deficiencies (sex hormones, growth hormone, and thyroid hormone), hypomagnesaemia, and endothelial dysfunction. We also described the development of these latter disorders in cancer patients following curative treatment. Based on the co-occurrence of metabolic disorders on the one hand and of hormonal
deficiencies, hypomagnesaemia, and endothelial dysfunction on the other in survivors of cancer, we hypothesized that these latter disorders play an etiologic role in the development of the metabolic syndrome and, thus, increase the risk of cardiovascular disease.

In chapter 3, we actually tested part of this hypothesis in long-term survivors of testicular cancer. Chemotherapy had been previously associated with an increased risk of either overt or compensated (normal total testosterone, but elevated luteinising hormone levels) Leydig cell dysfunction. We wondered whether the secretion of other hormones was disturbed as well and whether hormonal disturbances were associated with the metabolic syndrome in these men. We included 86 testicular cancer patients who had been cured with unilateral orchidectomy and cisplatin-based chemotherapy and did not use hormonal replacement therapy. These patients were compared with 44 testicular cancer patients with stage I disease who had been treated with orchidectomy only and with 47 healthy men of comparable age. Presence of the metabolic syndrome was determined using guidelines from the National Cholesterol Education Program ATP III. Thyroid-stimulating hormone, follicle-stimulating hormone (FSH), inhibin B, luteinising hormone (LH), total testosterone, sex-hormone binding globulin, free testosterone, oestradiol, dehydroepiandrosterone sulfate, and insulin-like growth factor 1 were determined in blood. Furthermore, cortisol metabolite excretion was measured in urine. The metabolic syndrome was present in 22 (26%) chemotherapy patients, in 16 (36%) stage I patients and in four (9%) controls. Compared with healthy controls, we found increased levels of LH, FSH and oestradiol, and decreased levels of inhibin B and total and free testosterone in patients treated with chemotherapy. Levels of adrenal, thyroid, and pituitary hormones did not differ. Total testosterone levels were lower and urinary cortisol metabolite excretion was higher in chemotherapy-treated patients with the metabolic syndrome than in those without. Chemotherapy-treated patients with the metabolic syndrome also had a higher body mass index (BMI) before treatment and a larger increase in BMI after treatment. Since total testosterone and urinary cortisol metabolite excretion were also associated with BMI after treatment, these hormones may, through this association, play a role in the development of the metabolic syndrome and cardiovascular disease in testicular cancer survivors.

Cardiovascular disease in testicular cancer survivors seems to develop after more than ten years of follow-up especially. Detection of risk factors and still asymptomatic vascular alterations at an earlier stage may enable timely
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intervention with the aim to prevent cardiovascular events after longer follow-up. In chapter 4, we investigated vascular structure and function to find out whether early signs of atherosclerosis are already present in survivors of testicular cancer during the first ten years of follow-up. We compared 90 chemotherapy-treated testicular cancer patients (median follow-up seven years) with 44 patients treated with orchidectomy only and with 47 healthy men. The same groups of patients and controls were described in chapter 3. We measured 24-h urinary albumin excretion and plasma levels of fibrinogen, high-sensitivity C-reactive protein (hs-CRP), von Willebrand factor (vWF), plasminogen activator inhibitor type 1 (PAI-1), and tissue-type plasminogen activator (t-PA). Furthermore, we assessed the intima-media thickness of the carotid artery, the distensibility, compliance and stiffness of the carotid artery, and the flow-mediated vasodilation of the brachial artery as measures of large artery structure and (endothelial) function. Microalbuminuria, a sign of generalised endothelial dysfunction, was present in ten (12%) chemotherapy-treated patients. The prevalence of microalbuminuria in these patients was significantly different from that in controls (0%) and in 3,348 men (age range 28-65 years) from a Dutch population study, in whom microalbuminuria prevalence was 4.6% (95% confidence interval for difference in proportions 0.3% - 13.7%). We also found higher levels of fibrinogen, hs-CRP, vWF, PAI-1 and t-PA in chemotherapy-treated patients than controls. The difference in PAI-1 between chemotherapy-treated patients and controls was much larger than the difference in t-PA. Furthermore, chemotherapy-treated patients with an elevated PAI-1 concentration (25 out of 90 patients) showed clustering of cardiovascular risk factors resembling the metabolic syndrome. Measurements of large artery structure and endothelial function did not differ between groups. The higher prevalence of microalbuminuria is an important finding in this survivor population, since microalbuminuria is a predictor of cardiovascular events. Furthermore, the increased concentrations of hs-CRP and the increased ratio of PAI-1 to t-PA suggest an inflammatory state and a decrease in fibrinolytic potency. Although these biochemical disturbances were not (yet) reflected by changes in large artery function and structure, they point to an increased atherosclerotic risk.

Besides vascular function, chemotherapy may affect cardiac function. In chapter 5, we investigated left ventricular and cardiac autonomic function to find out whether cardiac dysfunction contributes to an increased cardiovascular risk in testicular cancer patients following treatment with chemotherapy. Again, 90 chemotherapy-treated patients were compared with 44 stage I
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patients and 47 healthy controls. We measured plasma levels of N-terminal pro-brain natriuretic peptide, 24-h ambulatory blood pressure, baroreflex sensitivity, and cardiac left ventricular function using Doppler echocardiography. We found that chemotherapy-treated patients had a lower ratio of early and atrial filling velocities across the mitral valve (E/A-ratio) than stage I patients and healthy men. The lower E/A-ratio was not only associated with age and hypertension, but also with chemotherapy treatment. Natriuretic peptide levels were normal. Baroreflex sensitivity was similar in the three groups. The decrease in E/A-ratio may indicate impaired relaxation of the left ventricle. Although the importance of this finding with respect to the risk of cardiovascular disease is unknown, risk factors known to be associated with diastolic dysfunction should be treated.

Although direct damage from cytostatic agents to the vasculature has been proposed as a mechanism behind the development of acute and late cardiovascular disease in patients with testicular cancer, it was unknown whether standard vascular tests can be used to detect acute chemotherapy-induced vascular alterations. In chapter 6, we reported the results of a prospective study, in which we performed measurements of vascular and autonomic function in 65 patients with testicular cancer (median age 27 years, range 18-48 years) prior to and shortly following cisplatin-based chemotherapy. Before and within ten weeks after completion of chemotherapy, we measured platelet numbers, plasma levels of fibrinogen, vWF, PAI-1 and t-PA, plasma thrombin activatable fibrinolysis inhibitor (TAFI) activity, 24-h ambulatory blood pressure, baroreflex sensitivity, intima-media thickness of the common carotid artery, and flow-mediated vasodilation of the brachial artery. During treatment, two patients developed a myocardial infarction; at baseline, these patients had platelet numbers and levels of vWF, t-PA and fibrinogen above the upper limit of normal, while platelet numbers and vWF levels increased further during treatment. Overall, we found a significant increase in the plasma concentration of vWF, but no changes in fibrinogen, PAI-1, t-PA, and TAFI. Furthermore, the intima-media thickness of the common carotid artery increased significantly and more than might be expected from the natural course. Flow-mediated vasodilation and baroreflex sensitivity did not change. Increases in plasma vWF levels and carotid wall thickness may indicate chemotherapy-induced vascular damage and be of prognostic significance for the development of vascular complications in the long term.

Chemotherapy-related vascular toxicity may result from direct damage to endothelial cells. While the endothelial lining of the vasculature is normally involved in regulating vascular tone, and is crucial for maintaining homeostasis in the microcirculation; it was investigated to determine whether chemotherapy treatment induced endothelial damage. In chapter 6, we investigated the effects of chemotherapy on endothelial cells using human microvascular endothelial cells (HMEC-1). We found that chemotherapy-induced ICAM-1 expression was not induced in HMEC-1 following exposure to bleomycin and dextrazoxane, but chemotherapy-induced ICAM-1 expression may result in upregulation of bleomycin and contribute to chemotherapy-induced vascular damage.

Bleomycin is a standard B drug for testicular cancer, and potentially may result in pulmonary toxicity. Known risks include pneumonitis, and free radical-mediated lung damage. The long-term survival of patients with testicular cancer is high, and chemotherapy-related toxicity may have important implications for quality of life and long-term survival.
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involved in the maintenance of a normal vascular tone, a low inflammatory state, and a balance between thrombotic and fibrinolytic factors, bleomycin- and cisplatin-induced damage to endothelial cells may disturb these homeostatic functions. In chapter 7, we used the immortalized human dermal microvascular endothelial cell line HMEC-1 as an in vitro model to investigate the effects of bleomycin and cisplatin on the proliferation and apoptosis of endothelial cells and on their expression of intercellular adhesion molecule-1 (ICAM-1) and the fibrinolytic proteins PAI-1 and t-PA. Furthermore, we investigated potential modulation of cytotoxicity by amifostine and dexrazoxane. Endothelial cell survival decreased 50% after 0.3 μg/ml bleomycin and 2.6 μM cisplatin, respectively, which coincided with the induction of apoptosis. At high concentrations only, amifostine protected HMEC-1 from bleomycin-induced cytotoxicity, while dexrazoxane protected HMEC-1 from both bleomycin- and cisplatin-induced cytotoxicity. Since both modulators were also cytotoxic at high concentrations, absolute cell survival was not increased. Both bleomycin and cisplatin induced upregulation of ICAM-1 expression. PAI-1 was upregulated by bleomycin, while t-PA was upregulated by both bleomycin and cisplatin. These results indicate that bleomycin and cisplatin may damage and/or activate endothelial cells, which may result in the initiation or promotion of inflammation and atherothrombosis and contribute to the development of vascular events in patients treated with these cytostatic agents.

Previous studies demonstrated an increased risk for cardiovascular disease following treatment for testicular cancer. Unfortunately, only cardiovascular mortality, not morbidity, was investigated in the two largest studies, while cardiac morbidity can have a serious impact on the quality of life of long-term survivors and is likely to be of poor prognostic significance. In chapter 8, we argued that assessment of cardiac mortality only may result in an underestimation of the importance of cardiac disease as a long-term complication of treatment, since many cases of cardiac disease may be nonfatal (initially). This holds true for the determination of cardiac disease risk in long-term survivors of various malignancies, including testicular cancer.

Bleomycin has proved to be essential for the high treatment efficacy of standard BEP chemotherapy. However, use of bleomycin is limited by its potentially life-threatening pulmonary toxicity. This bleomycin-induced pneumonitis (BIP) is initiated by damage to the lung vasculature by cytokines and free radicals and can, thus, be viewed as a vascular toxic effect as well. Known risk factors for bleomycin-induced pneumonitis have proved to be
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inadequate in predicting pulmonary toxicity in the individual patient. Bleomycin is mainly excreted by the kidneys, but may also be inactivated by bleomycin hydrolase. In chapter 9, we investigated the relation between an A1450G polymorphic site in the bleomycin hydrolase gene and the development of pulmonary toxicity in patients with a disseminated germ cell tumour following bleomycin-containing chemotherapy in an attempt to identify a novel tool for predicting an increased risk of pulmonary toxicity in the individual patient. Data regarding the development of BIP and the presence of known risk factors for BIP were derived from the medical records and the bleomycin hydrolase genotype was determined using polymerase chain reaction and a restriction fragment length polymorphism technique. In 340 patients (81% of all testicular cancer patients treated with bleomycin between 1977 and 2003), BIP was present in 38 (11%) patients and was fatal in four of these cases. Patients with BIP had a higher age and a lower pretreatment creatinine clearance than patients without BIP. The frequencies of BIP were 14%, 8% and 14% in patients with the A/A, A/G or G/G genotype, respectively. Therefore, no clear association was found between the bleomycin hydrolase genotype and the risk of pulmonary toxicity.

Finally, in chapter 10, we have indicated how the results of the above-mentioned studies fit into current knowledge of chemotherapy-induced vascular toxicity. Because preventive measures are (still) lacking, we have further recommended early identification of late vascular toxicity through periodic screening.